Universidade de Aveiro Departamento de Biologia
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Maria João Maia Rocha de Almeida

Processos de pré- e pós-assentamento que regulam o recrutamento do caranguejo verde *Carcinus maenas*

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tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Prof. Dr. Henrique de Barros Brito Queiroga, Professor Auxiliar com Agregação do Departamento de Biologia da Universidade de Aveiro.

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palavras-chave

Carcinus maenas, megalopas, juvenis, Ria de Aveiro, Rio Mira, assentamento, recrutamento, *upwelling*, *downwelling*, densidades naturais, *Zostera noltii*, taxas de crescimento em juvenis, locomoção, canibalismo.

resumo

O sucesso do recrutamento em espécies com ciclos de vida complexos, i.e. com duas ou mais fases de desenvolvimento, depende do fornecimento de larvas aos locais apropriados do assentamento (mecanismos de pré-assentamento) e na consequente sobrevivência destas larvas até aos estágios bentónicos iniciais (mecanismos de pós-assentamento).

O estudo dos principais processos físicos que controlam a variabilidade intra-anual do recrutamento do caranguejo Carcinus maenas foi possível com à obtenção em séries diárias da abundância de megalopas no plâncton e do seu assentamento em substratos artificiais de assentamento, e de parâmetros físicos relacionados com a amplitude de maré e o upwelling, durante os meses da época reprodutiva deste caranguejo. Os nossos resultados demonstraram um padrão cíclico de fornecimento de megalopas de C. maenas aos estuários na costa ocidental de Portugal, relacionado com a amplitude de maré e favorecido pelos ventos de sul. O assentamento de megalopas nos substratos artificiais de assentamento mostrou-se desacoplado do fornecimento nos dois estuários. Os dados obtidos sugerem que as megalopas são transportadas para a costa após a acção de ventos de downwelling, e o fornecimento para os estuários ocorre por transporte selectivo por corrente de maré.

A utilização de substratos artificiais para medir densidades de juvenis de Carcinus maenas deve ser ponderada, uma vez que se verificou que em habitats estruturalmente menos complexos os juvenis utilizam os colectores como refúgio; em habitats mais complexos, os juvenis preferiram refugiar-se nas ervas marinhas existentes. No entanto, a estimativa de abundâncias diárias medidas nos colectores permitiu a descrição das abundâncias dos vários estádios de desenvolvimento juvenis, bem como as respectivas taxas de crescimento.

As capacidades locomotoras de diferentes classes de tamanho de juvenis de C. maenas foram estimadas mediante o cálculo de um índice de locomoção. Os nossos resultados sugerem que os juvenis mais pequenos estão relativamente protegidos em habitats com vegetação densa, provavelmente porque este tipo de habitats inibe os movimentos dos juvenis de maiores dimensões. Foram também encontradas evidências da existência de segregação temporal na actividade locomotora das diferentes classes de juvenis, que funcionará como um mecanismo que permite reduzir o canibalismo e, consequentemente, aumentar a capacidade de suporte dos habitats juvenis.

Foi realizado um estudo sobre o canibalismo nos juvenis de C. maenas na Ria de Aveiro, in situ e utilizando densidades reais. Verificou-se que as presas mais pequenas eram as mais vulneráveis, sendo os juvenis de maiores dimensões os predadores mais eficazes. Por outro lado, as taxas de canibalismo foram menores em habitats com elevadas densidades de refúgio (Zostera noltii). A estimativa das taxas diárias de mortalidade devidas ao canibalismo são aproximadamente metade das taxas diárias de mortalidade devidas aos processos de pré-assentamento, indicando que esta população de C. maenas será regulada pelo fornecimento larvar, i.e., será uma população regulada essencialmente por mecanismos pré-assentamento.

keywords

Carcinus maenas, megalopae, juveniles, Ria de Aveiro, Rio Mira estuary, settlement, recruitment, upwelling, downwelling, intertidal habitats, *Zostera noltii*, natural densities, juvenile growth rates, locomotory activity, cannibalism.

abstract

The success of recruitment in complex life cycle species, i.e. with two or more developmental phases, is dependent on the supply of larvae to the appropriate settlement sites (pre-settlement processes) and on the subsequent survival of these larvae through the early benthic stages (post-settlement processes).

The main physical processes that control intra-year variability of recruitment in the portunid crab *Carcinus maenas* were studied by obtaining daily series of abundance of megalopae in the plankton, settlement of megalopae on bottom-deployed collectors, and physical parameters related to tide amplitude and upwelling, during several months within the reproductive season. Our results demonstrated a cyclic pattern of supply of *Carcinus maenas* megalopae to estuaries on the west coast of Portugal, related to the tide amplitude. Furthermore, supply was enhanced by southerly winds. Settlement of megalopae on artificial settlement substrates deployed on the bottom was uncoupled to supply at both estuaries. Our results suggest that transport of *C. maenas* megalopae to the nearshore is accomplished by onshore advection following downwelling winds, and supply to the estuaries occurs by selective tidal stream transport.

The use of artificial collectors to measure juvenile shore crab densities must be carefully considered as juveniles appear to use of the collectors as refuges in less complex habitat, while in more complex habitats crabs preferred to take shelter among the naturally-occurring seagrasses. Nevertheless, artificial collectors deployed daily allowed the description of the different juvenile instar abundances over time and the estimation of juvenile growth rates.

The locomotory activity of different size classes of shore crab juveniles were assessed through the estimation of a locomotory index. Our results suggest that small juveniles are relatively protected under dense vegetation cover due to lower mobility of larger crabs, and evidence temporal segregation of activity windows between juvenile crabs of different size, which may be a key mechanism to reduce cannibalism and therefore increase carrying capacity of nursery habitats.

Cannibalism in *C. maenas* juvenile shore was measured *in situ* and at natural densities. Smaller preys were the most predated and medium and large size of juveniles was the most successful predators. Also, rates of cannibalism diminished at higher densities of refuge (the seagrass *Zostera noltii*). In the case of Ria de Aveiro, the average daily mortality rates due to cannibalism is approximately half of the mortality caused by pre-settlement processes. These results suggest that this shore crab population is mainly regulated by larval supply, i.e., it is a pre-settlement regulated population.

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Chapter 1

General introduction

General introduction

Many marine benthic organisms possess complex life histories with two developmental phases, in which demersal adult stages produce larvae that develop in the pelagic environment before recruiting to the benthos (Roughgarden et al. 1988, Ólafsson et al. 1994, Swerear et al. 2002). Most commercially exploited marine decapod crustaceans, such as lobsters, crabs and shrimps, have complex life histories. In the broadest sense, recruitment is defined as the addition of new individuals to populations or to successive life-cycle stages within populations (Caley et al. 1996). Recruitment to the adult population involves several steps: larval development, dispersal during development, larval supply to suitable settlement habitats, settlement (i.e. the transition from a planktonic to a benthic existence) and survival and juvenile development. A variety of physical and biotic processes influence the success of recruitment both before and after settlement. Usually, these processes can be divided into: (1) pre-settlement processes, operating from the time of hatching to initial larval settlement, and (2) post-settlement processes, operating for the remainder of benthic life from early juvenile stages through adulthood (Shanks 1995, Wahle 2003).

Pre-settlement processes

During the time spent in the plankton, larvae of benthic species with a two-stage life cycle can be dispersed by forcing agents through the coastal waters but, at the end of the planktonic development, larvae must find the nursery habitats where they will recruit to the adult population (Epifanio 1995, Morgan 1995). Physical processes that force transport of planktonic larvae of invertebrates are responsible for some of the spatial and temporal variability in recruitment.

Generally it is accepted that mechanisms that rule cross-shelf dispersal and return migration of larvae are separated in 2 steps (Boehlert and Mundy

1988, Shanks 1995): (1) the transport of the larvae from the shelf to the coast and (2) the passage through inlets and upstream movement until an appropriate environment to settlement is found. In each of these steps, environmental forcing interacts with larval behaviour to produce what marine biologists call a "recruitment mechanism" (Queiroga and Blanton 2004).

Across-shelf transport

Some of the physical processes acknowledged to be able to transport larvae onshore are: wind-generated superficial currents (including sea breezes and Langmuir circulation), wind-drift currents and Ekman transport, onshore convergence following relaxation of upwelling favourable winds, residual tidal currents, internal waves or density-driven flow (reviewed by Shanks 1995). In all these processes, the rate and direction of transport depends critically on the time of occurrence and depth distribution of larvae, which interacts with the vertical structure of the flow field (Blanton et al. 1995, Queiroga and Blanton 2004).

Wind-driven circulation and tides are two of the most important physical processes accounting for the variability of supply of larvae to littoral and estuarine systems (Epifanio and Garvine 2001). Also buoyancy-induced currents, associated with mass of waters with differences in density (e.g. outflow of estuaries, surface heat flux or the interaction of coastal and oceanic water masses at the seaward margin of the shelf) contribute to onshore larval transport (Queiroga and Blanton 2004). The relative importance of these agents varies geographically, and different shelf areas around the world have been categorized as wind-dominated, tidally dominated or buoyancy-dominated (Epifanio and Garvine 2001).

On most shelves the circulation driven by wind stress acting directly on the water surface is the most energetic component (Epifanio and Garvine 2001). In the case of eastern boundaries of the North Pacific and the North Atlantic, wind forcing has been recognized as an important agent in the shoreward transport of crustaceans larvae namely in barnacles (Farrel et al. 1991,

Roughgarden et al. 1991, Cruz 2000) and crabs (Goodrich et al. 1989, Little and Epifanio 1991, Blanton et al. 1995, Paula et al. 2001, Almeida and Queiroga 2003). Equatorward winds in the northern hemisphere are responsible for upwelling transport, i.e., divergence of the surface Ekman seaward and consequent convergence of nutrient-rich bottom waters shoreward. The resulting production is distributed in the vicinity of upwelling fronts and large concentrations of larvae can often congregate there (Shanks et al. 2000). Onshore convergence of the surface layer, and subsequent transport of larvae, occurs during the relaxation (or reversal) of upwelling favourable winds, and compensating downwelling of water close to the coast. Therefore cross-shelf transport of larvae will depend on their vertical position: during upwelling larvae that remain in the upper boundary layer suffer offshore transport; on the contrary the transport will be onshore if larvae are located in the bottom layer. Recent studies have gather evidences that during upwelling events decapod larvae at the Portuguese north coast exhibit vertical migrations synchronized with the day cycle (diel vertical migration, DVM; Queiroga et al. 2006, dos Santos et al. 2008). This behaviour enhances retention over the shelf, increasing the likelihood of onshore and up-estuary transport by a variety of bio-physical processes (Marta-Almeida et al. 2006). It has been observed the reverse situation during downwelling (Blanton et al. 1995, Olmi 1995, Queiroga 1996, Queiroga 2003).

Internal tidal waves, resultant form the interaction of the barotropic tidal circulation on the continental shelf, have attracted attention as a potential mechanism for across-shelf transport of larvae (Shanks 1995). In thermally stratified waters, the flow over topographic features of the bottom produces waves in the thermocline that can be topographically trapped. At slack tide the waves are released and progress towards the coast. It has been proposed that neustonic organisms and other materials at the surface can be trapped and carried by the convergence zone that develops at the trailing edge of the waves and carried across the shelf (Shanks 1983, 1988). Since the formation of internal waves is related to tidal range, this transport mechanism would result in fortnight pulses of supply and settlement into coastal habitats.

Supply into estuaries

After being transported to the near-shore environment, estuarine larvae must find the estuarine inlet and travel upstream in order to find an appropriate settlement location. Because planktonic larvae are unable to swim against the net seaward flow of estuarine waters, upstream movement must be accomplished through any kind of adaptation that opposes the net downstream flux of waters (Boehlert and Mundy 1988, Epifanio 1988). Such adaptation is selective tidal stream transport (STST; Olmi 1994, Queiroga et al. 1994, Queiroga 1998). Basically STST consists in synchronous vertical migration of the megalopae stage from the bottom to the overlying water during flood tide and settlement to the bottom during the following ebb tide.

Decapod larvae can regulate their swimming activity, and therefore modify their vertical distribution in the water column through responses to specific environmental stimuli or according to endogenous cycles of activity.

Post-settlement processes

The study of post-settlement processes for populations and community dynamics is presently a major focus in marine ecology. Settlement and early juvenile stages are known to be critical periods in the life cycle of benthic organisms because of very high mortality (Gosselin and Qian 1997, Hunt and Scheibling 1997). In fact, the transition form a planktonic larval phase to benthonic juvenile life frequently produces bottleneck effects on the number of individuals, affecting the dynamics of juvenile and adult populations (Eggleston and Armstrong 1995, Moksnes 2004). The most relevant processes affecting the abundance and distribution patterns of settlers and early benthic juvenile stages are: (1) selection of settlement habitat and (2) habitat-specific post-settlement processes (e.g. dispersal, survival and growth; Caley et al. 1996).

Selection of settlement habitat

Once in shallow areas settlement of late larvae depends on the recognition of environmental cues at the appropriate areas. These cues can be physico-chemical, such as light intensity, surface chemistry, texture or complexity of the substrate (Welch et al. 1997), or biological, as the presence or absence of conspecifics, competitors and predators (O'Connor 1993, Paula et al. 2003). In lack of the right cues, competent megalopae show plasticity in the duration of the stage and they can shorten or delay settlement and metamorphoses until appropriate settlement habitats are encountered (Wolcott and DeVries 1994). The active habitat selection can significantly affect settlement patterns and decouple the relationship between larval supply and recruitment (Moksnes and Wennhage 2001).

High densities of juvenile decapods are frequently found in specific microhabitats with high structural complexity that offers protection from predation by fish and cannibalism by conspecifics (e.g. dungeness crabs *Cancer magister* in oyster shell: Fernandez et al. 1993, Eggleston and Armstrong 1995; fiddler crabs *Uca pugnax* and *U. pugilator* in marshes: O'Connor 1993; red king crab *Paralithodes camtschaicus* in rocky cobble: Loher and Armstrong 2000; blue crab *Callinectes sapidus* in seagrass beds: Moksnes et al. 1997, Orth and van Montfrans 2002; shore crab *Carcinus maenas* in mussel beds, shell debris, filamentous macroalgae and eelgrass beds: Moksnes 2002).

Habitat-specific post-settlement processes

Juvenile mortality may obscure the effects of variation in larval settlement through both density density-independent and density-dependent mortality of juveniles.

Predation is often a dominant component of density-dependent mortality for juveniles that can modify initial settlement patterns, decoupling its relation with the subsequent population size. (Hunt and Scheibling 1997). Cannibalism, defined as intraspecific predation, can comprise a major source of predation upon juveniles that regulates recruitment success in size-structured populations

typical of many aquatic systems (Luppi et al. 2001, Moksnes et al. 1997, Moksnes 2004, Sainte-Marie et La France 2002, Stevens and Swimney 2005).

Predation rates are often mediated through utilization of refuges which may include spatial (habitat) and/or temporal (diurnal, seasonal, annual) components that reduce access or effectiveness of predator impact on prey. Spatial and temporal aspects of refuges may interact so that juvenile mortality may exhibit significant temporal variation within a refuge habitat (Hines and Ruiz 1995).

Post-settlement movements of juveniles are another important process for local population dynamics that can alter predator-prey and competition interactions, by mitigating crowding effects (Moksnes 2002). Actually, density-dependent effects can be altered, at least in motile species, by allowing the escape from risky areas. Nevertheless, this effect is often ignored or minimized in post-settlement processes experiments, and often, estimates of mortality are confounded by dispersal-related losses (Palmer et al. 1996, Moksnes and Wennhage 2001).

The model species: *Carcinus maenas*

The shore crab *Carcinus maenas* is native to the Atlantic coast of Europe and northern Africa, from Norway to Mauritania. It is a decapod species eurythermal and euryhaline, found in a variety of habitats, including protected rocky shores, cobble beaches, sandflats and tidal marshes. Its large ecological plasticity combined with its aggressive competitive and predatory behaviour, made it a successful global invader, with established reproductive populations on both the east and west coast of North America, South Africa, Patagonia, Australia and Tasmania (Carlton and Cohen 2003, Yamada et al. 2005). Its widespread distribution, abundance in coastal and estuarine waters and its ecological important role constitute major reasons for its frequent use in experiments.

The growth, reproduction and length of time required for development for green crabs depends upon temperature and salinity conditions, as well as food availability. Females can mate multiple times within a single year, and females may produce more than one clutch per year (Yamada et al. 2005). The number of eggs per reproductive event varies by female size, and a typical female will produce 185,000 or more eggs/event. Eggs are brooded externally by the female and hatch into plankton larvae after 17-80 days. Larvae develop through 4 successive zoeal stages and then metamorphose into benthic dwelling, postlarval crabs (reviewed by Queiroga and Blanton 2004).

Hatching in the portunid shore crab *Carcinus maenas* (L.) occurs in the lower parts of the estuaries on nocturnal ebb tides during spring and early summer (Zeng & Naylor 1997, Queiroga et al. 1994). Newly hatched first zoea is quickly transported seawards by selective tidal stream transport (Queiroga et al. 1994, 1997). Most of larval development takes place in the ocean, within 4 to 6 wk depending on temperature (Nagaraj 1993), before the reinvasions of estuarine waters by the megalopae (Queiroga et al. 1994). This stage is more abundant in estuarine waters during night-time flood, and again uses selective tidal stream transport, but this time to move upstream against the net seaward flow of estuarine waters (Queiroga 1998). It appears that the megalopae transport to the shore is associated to the relaxation of the north winds, during which the superficial coastal water converges to the shore and can transport this larval stage (Queiroga 1996, Almeida and Queiroga 2003).

The competent *C. maenas* megalopa actively select settlement sites (Hedvall et al. 1998), which are usually intertidal structurally complex habitats such as mussel beds, filamentous macroalgae or seagrasses (Thiel and Dernedde 1994, Moksnes 2002, Paula et al. 2006, Silva et al. 2006). Juvenile crabs remain in high densities in such habitats, which are known to provide adequate shelter from predation for these early stages (Moksnes et al. 1998, Moksnes 2002). Different juvenile cohorts co-exist and cannibalism may greatly reduce the density of early recruits (Moksnes 2004).

One of the most interesting findings on shore crab recruitment processes in the Mira Estuary (SW Portugal) is that maximum settlement occurs around

neap tides, clearly decoupled from supply (Paula et al. 2006, Queiroga et al. 2006).

Aims of the work and structure of the thesis

In a general way, the main objectives of the present thesis were to understand the factors that affect larval supply and settlement and investigate some processes that regulate post-larvae recruitment of the portunid crab *Carcinus maenas*. The thesis is structured into several chapters, each one representing different units with specific objectives, incorporated in the global aim of the study. Every chapter is independent and contain its individual Introduction, Methods, Results, Discussion and References section. This structure is very similar to the publishing layout and facilitates the reading and understanding of the contents. Each chapter corresponds to two already published papers, a submitted article and a manuscript. The candidate was fully involved in the planning, in sampling, in all laboratory processes, data analysis and discussion of the results of all the works. In the particular case of the paper presented in Chapter 2, this is true only for the data acquired at the Ria de Aveiro estuary, which is the reason why the candidate is not the first author of the article.

In Chapter 2 (*Tide and wind control of megalopal supply to estuarine crab populations on the Portuguese west coast*) it is described a study were the temporal and spatial patterns of invertebrate larval supply and settlement in estuaries on the Portuguese west coast were investigated. Moreover, the main physical processes that control intra-year variability of recruitment in the shore crab were identified. The efficiency of artificial substrate collectors according to habitat complexity in estimating densities and growth rates of the shore crab juveniles was assessed through the study presented in Chapter 3 (*Use of artificial substrate collectors in estimating densities and growth rates in shore crab juveniles*). Chapter 4 (*Effect of crab size and habitat type on the locomotory activity of juvenile shore crabs, Carcinus maenas*) describes a study

that examined the habitat specific distribution of *Carcinus maenas* juveniles in Rio Mira estuary. The locomotory capacities in different juvenile size classes and the influence of day and lunar cycles on the mobility of each size class were also examined. In Chapter 5 (C*annibalism among Carcinus maenas juveniles measured in situ*) it is described a study where a field experiment was conducted in order to assess the influence of cannibalism on the survival of juveniles using cages deployed in the field. The bulk of the thesis is preceded by this General introduction, where the main and specific objectives are explained and the current state of the art in the research field is described. The last section of the thesis, Final remarks, summarizes in a comprehensive global and integrated the importance of the main results obtained during this work.

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Chapter 2

Tide and wind control of megalopal supply to estuarine crab populations on the Portuguese west coast

Tide and wind control of megalopal supply to estuarine crab populations on the Portuguese west coast

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ABSTRACT

Physical processes that force transport of planktonic larvae of invertebrates are responsible for some of the spatial and temporal variability in recruitment. We investigated the influence of tide- and wind-driven circulation on intra-year variability of megalopal supply to populations of the crab *Carcinus maenas* in 2 estuaries on the Portuguese west coast. Daily data on physical variables and on supply and settlement of megalopae were subjected to time series analysis and multiple regression techniques in order to identify periodicity in the variables as well as time lags between larval supply and physical variables. Relaxation of northerly winds, which favour upwelling, was associated with temperature increase and subtidal sea level rise at the coast, which are indicative of coastal convergence of the surface layer. Supply of megalopae to the estuaries, as measured with passive plankton nets, followed a fortnightly cycle with maximum larval supply at the time of maximum tidal amplitude. Supply was enhanced by southerly winds, with delays of 0 to –2 d. Settlement of megalopae on artificial settlement substrates deployed on the bottom was uncoupled to supply at both estuaries. Therefore, transport of *C. maenas* megalopae to the nearshore is accomplished by onshore advection following downwelling winds, and supply to the estuaries occurs by selective tidal stream transport. Involvement of internal waves and internal tidal bores cannot be ruled out, but very particular periodicities of the generating mechanisms would have to be assumed to account for the observed time lags.

Keywords: *Carcinus maenas*; larvae; recruitment; upwelling; tides.

Introduction

Tides and wind-driven circulation are 2 of the most important physical processes that account for the variability of supply of larvae to littoral and estuarine systems. The current paradigm holds that recruitment of estuarine species with a dispersive larval phase is a 2-step process (Boehlert and Mundy 1988, Shanks 1995): (1) onshore transport of larvae over the shelf and (2) passage through inlets and upstream movement in search of a suitable habitat for settlement. These studies emphasize the role of active behaviour of larvae, principally the vertical position in the water column, in the control of horizontal transport. Because the environmental processes that dominate neritic and estuarine waters differ, different behavioural mechanisms must be activated during the 2 steps. The following discussion on potential transport processes concern crustacean larvae, but similar evidence regarding fish also exists (e.g. Boehlert and Mundy 1988, Epifanio and Garvine 2001, Sponaugle et al. 2005).

Tides are the driving force that causes internal waves. In thermally stratified waters, tidal currents flowing over the shelf break cause waves in the thermocline that are topographically trapped. At slack tide the waves are released and progress towards the coast. Shanks (1983, 1988) has proposed that internal waves can transport neustonic larvae across the shelf, at the convergence zone that is entrained by the internal waves. Since formation and propagation of internal waves is related to tidal amplitude (but not necessarily during spring tides; see Pineda 1994) this mechanism could result in pulses of supply of megalopae occurring at intervals of 2 wk. In linear internal waves, particles pass into and out of the convergence zone, and onshore transport does not occur (Franks 1997). However, in non-linear, strongly asymmetric internal waves, the speed of the surface current generated by the wave can be higher than the phase speed, resulting in an accumulation of particles at the front of the wave, and subsequent onshore transport (Pineda 1999, Helfrich and Pineda 2003). This mechanism can only operate provided larvae are neustonic, such as in the case of several decapod megalopae (Zeldis and Jillet 1982, Shanks 1985). Some estimates point to long-term mean onshore transport velocities of 0.005

m.s–1, 2 orders of magnitude below the phase speed of the wave, suggesting that they may not be significant for crossshelf transport of larvae (Epifanio and Garvine 2001).

As internal waves approach shallow waters they break in a similar way as surface waves do, causing internal tidal bores. Pineda (1991, 1994) proposed the hypothesis that shoreward-breaking internal waves produce nearshore upwelling and transport subsurface water to the shore, carrying with it water-column larvae residing below the thermocline. Internal bores may also produce onshore flow at the surface, following the downwelling and seaward flow of the denser water that was previously advected to the surface. In this case, neustonic larvae would be transported shoreward (Pineda 1994). Both mechanisms evoking the role of internal tidal bores would also result in pulses of larval supply occuring at intervals of 2 wk. However, internal bores do not necessarily occur around spring tides, as shown by Pineda (1991, 1995). Internal waves may have larger amplitude than surface waves, and therefore may break at considerable distances from the shore. The scale of the shoreward transport is not known, but it is likely that internal bores may control supply of larvae only in shallow nearshore waters (Pineda 1994).

Wind-driven circulation has been implied in the cross-shelf transport of barnacle and decapod larvae in eastern boundaries of the North Pacific and North Atlantic, which are dominated by upwelling (Roughgarden et al. 1991, Almeida and Queiroga 2003). At these types of coasts, onshore transport of larvae takes place during the relaxation of the equatorward winds, which causes onshore convergence of the surface layer and collision of upwelling fronts with the coast. Differences in upwelling strength along the coast (Connolly et al. 2001), as well as alongshore flow resulting from intensification and relaxation of upwelling-favorable winds (Wing et al. 1995), may also result in temporal and spatial variation of larval supply and affect recruitment at regional and local scales. Episodic wind events with return periods of weeks to months that result in coastal increase of subtidal sea level (SSL) and storm surges (such as the passage of fronts and hurricanes), have been implied in augmented supplies of crab megalopae to estuarine systems (Goodrich et al. 1989). On a regional scale,

the opposing effects of near-coast buoyancy flow provided by river discharge and wind-driven circulation further offshore may provide a mechanism for retention of larvae, as indicated by several studies on the western North Atlantic concerning fish and decapod larvae (Epifanio and Garvine 2001). Down-the wind transport of barnacle larvae has also been implicated as the mechanism underlying the correlations between onshore wind intensity and settlement of barnacle cypris (Hawkins and Hartnoll 1982).

Other physical mechanisms for cross-shelf transport of larvae have also been proposed (reviewed by Shanks 1995), such sea breezes (see Tapia et al. 2004), Langmuir circulation, and density-driven flow. However, the evidence supporting these mechanisms is considerably less extensive.

A clear conceptual background for upstream movement in estuaries and semi-enclosed bodies of water dominated by tidal circulation is emerging. Invertebrate and fish larvae migrate into estuaries using selective tidal stream transport (STST, reviewed by Forward and Tankersley 2001). During upstream STST, larvae settle on, or move close to, the bottom during ebb tide, to avoid being displaced seaward, and they ascend in the water column during flood tide. The best description of the bio-physical control of STST is available from a series of field and laboratory studies on crab megalopae, which are consistently more abundant in estuaries during night flood tides than at other times (e.g. Little and Epifanio 1991, Queiroga et al. 1994). This pattern arises from a combination of inhibition of swimming activity by high light intensities, ascent in the water column triggered by increases of hydrostatic pressure and salinity during flood, and sustained swimming in the presence of high turbulence levels. Chemical substances present in estuarine water, which result from the decomposition of organic matter, inhibit swimming during the day and cause sinking to the bottom (Forward and Rittschof 1994, Forward et al. 1997). This behavior may enhance concentration of megalopae close to estuarine inlets. As the tide begins to rise, the concurrent increase of pressure and salinity above species-specific threshold levels triggers an ascent in the water column that promotes the transport of the megalopae into the estuary (DeVries et al. 1994, Tankersley et al. 1995). Swimming is maintained during the whole duration of the flood tide by high

levels of turbulence, and during slack-after-high-water the megalopae stop swimming due to decreased turbulence levels and drop to the bottom, where they remain during the ensuing ebb tide, because the salinity drop overrides the effect of turbulence (Welch et al. 1999, Welch and Forward 2001). In some cases endogenous rhythms synchronised with the tide also assist in upstream STST (DeVries et al. 1994). The threshold rates of increase in pressure and salinity necessary to elicit the swimming response during flood (Tankersley et al. 1995) may be the basis for the increased abundance during spring tides and for the clear 2 wk pattern detected in meso-tidal systems (Moser and MacIntosh 2001, Paula et al. 2001), contrasting with the diffuse pattern detected in systems with a lower tidal range (e.g. van Montfrans et al. 1995). The larger the tidal range, the higher the pressure and salinity increase rates during flood, and the more likely crab megalopae respond by swimming from the bottom to the water column.

In the present study we investigated temporal and spatial patterns of invertebrate larval supply and settlement in estuaries on the Portuguese west coast. Our aim was to identify the main physical processes that control intra-year variability of recruitment in the portunid crab *Carcinus maenas*. The Portuguese coast has a semidiurnal tidal regime where the range of the tide averages 2 m and is subjected to seasonal upwelling (Wooster et al. 1976). *C. maenas* forms large populations in Portuguese estuaries and has a planktotrophic larval phase that develops in shelf waters, showing maxima of abundance in the middle shelf in northern Portugal (Queiroga 1996).

Our first hypothesis was that supply of megalopae should occur with a semilunar periodicity, because internal waves, internal tidal bores and STST are all related to tidal range. Our second hypothesis was that supply should increase following relaxation or inversion of upwelling-favorable equatorward winds, when coastal convergence of the surface layer takes place. We addressed these problems by obtaining daily series of abundance of megalopae in the plankton, settlement of megalopae on bottom-deployed collectors, and physical parameters related to tide amplitude and upwelling, during several months within the reproductive season, in 1 estuary in the northern (Ria de Aveiro) and

1 in the southern (Mira Estuary) part of the Portuguese west coast. In order to relate the change in abundance of megalopae inside the estuaries with shelf processes, observations of supply and settlement were conducted in the lower part of the estuaries, within 1 tidal excursion from the inlet.

Materials and Methods

Oceanography of the Portuguese west coast

The Portuguese coast is located on the northern limit of the eastern North Atlantic upwelling region, where most of the climate and coastal hydrology is controlled by the position of the Azores anticyclone that migrates northward during spring (Fiúza et al. 1982, Haynes et al 1993). Two typical current patterns can be described in the shelf/slope system. (1) During winter, the Iberian Poleward Current, which normally flows over the slope, is reinforced by strong southwesterly winds associated with the passage of cold fronts, and the northward flow extends to the inner shelf (Frouin et al. 1990, Haynes and Barton 1990, Haynes et al. 1993). Superimposed on the above regime, freshwater inflow from rivers (the Western Iberia Buoyant Plume) can force nearshore circulation in the neighborhood of estuaries. (2) In spring, a transition to equatorward flow occurs, typical of the upwelling regime driven by northerly alongshore winds. Northerly winds advect the surface layer offshore, causing a drop in sea level and a cooling of the surface water (Wooster et al. 1976, Fiúza 1984, Jorge da Silva 1992). The upwelling season typically lasts from April to September and has intensity maxima in July and August, although intense episodes can also happen during winter (Borges et al. 2003, Huthnance et al. 2002). Upwelling theory predicts that onshore convergence of the surface layer takes place as soon as equatorward winds relax (Bakun 1996); this does not require a complete reversal of the wind.

Larval biology of Carcinus maenas

The European common shore crab *Carcinus maenas* is a portunid species that forms large populations in Portuguese estuaries. In some cases, these estuaries sustain fisheries of local economic importance (Marques and Costa 1983, Gomes 1991, Sprung 1994). This crab is one of the best studied invertebrate species and has an extensive geographical distribution, which has been extended during the last century through artificial introduction to other continents followed by natural range expansion (Grosholz and Ruiz 1995, d'Udekem d'Acoz 1999, Thresher et al. 2003). First zoeae hatch during nightly ebbs of neap tides and are quickly flushed into coastal waters (Queiroga et al. 1994, 1997). This process is controlled by an endogenous rhythm of vertical migration in synchrony with the tide, which maximizes downstream transport (Zeng and Naylor 1996a, c, Duchêne and Queiroga 2001). Larvae take 4 to 6 wk, depending on water temperature, to reach the megalopa stage in shelf waters (Dawirs 1985, Nagaraj 1993). Larvae of the species have been found throughout the top 60 m of water, with abundance maxima located on the middle shelf (Queiroga 1996). The megalopal stage appears to be neustonic only during the night (Zeng and Naylor 1996b) and travels up the estuary during night flood tides using STST (Queiroga et al. 1994, Queiroga 1998, Zeng and Naylor 1996b). Previous studies of megalopae supply to the Ria de Aveiro using plankton nets (Queiroga 2003) and artificial collectors (Almeida and Queiroga 2003) detected a positive association with relaxation events of upwelling-favorable equatorward winds, but did not find any effect of tidal amplitude. The megalopae of *C. maenas* seem to settle in a variety of subtidal and intertidal estuarine habitats, showing a preference for those that are structurally complex (Paula 2006).

Sampling locations

The Ria de Aveiro is a shallow coastal lagoon on the northwest coast of Portugal with an average depth of 1 m (Fig. 2.1B, C). It can be considered a bar-built estuary according to the classification of Pritchard (1967). The inlet is artificially maintained by 2 long concrete and stone jetties approximately perpendicular to the coast line. The topography of the Ria de Aveiro is rather

complex, with 4 main channels that radiate from the inlet and several branches, islands and mudflats forming an intricate system that provides a good habitat for *Carcinus maenas*. The River Vouga is the most important of the rivers and smaller streams that discharge into the lagoon, accounting for 2/3 of the total freshwater input (Vicente 1985). Circulation in the lagoon is dominated by tides, except during periods of peak flow, which are coincident with heavy rainfall (Barrosa 1985, Dias 2001). Tides are semidiurnal with an average range of 2.1 m, and ranges of ca. 1 m and 3 m in extreme neap and spring conditions. The Canal de Mira (not part of the Mira Estuary), where sampling took place, runs SSW from the inlet for 25 km, parallel to the coast. This channel can be considered a small subestuary in itself. A headland that separates the inlet channel into 2 different arms diverts about 20% of the tidal prism of the flooding tide to the Canal de Mira. Other communications with the rest of the system are negligible. At the southern end, Canal de Mira receives a continuous freshwater supply from a small system of ponds and streams. In the area of the sampling stations the tides are slightly asymmetrical, with flood duration exceeding ebb duration by 25 min on average. The delay of the tide at the sampling sites relative to the inlet varies between 20 and 40 min, according to tidal amplitude and phase (Queiroga 1995). Salt marshes occur throughout the lower 10 km of the Canal de Mira, and intertidal areas with dense cover of seagrass meadows formed by *Zostera noltii* are common.

The Mira Estuary (as distinct from the Canal de Mira) is a morphologically simple estuary located on the southwestern Portuguese coast (Fig. 2.1D, E). The estuarine section extends about 40 km inland in a single channel. In the lower 2 km the estuary presents a flood delta composed of 2 main complexes of sandy shoals that emerge during spring low water. Maximum depth is around 20 m at the Vila Nova de Milfontes bridge (2 km from the mouth), and upstream the single channel is about 8 to 10 m deep, becoming shallower inland. The tide is semidiurnal with ranges of ca. 1.0 m at neap tide and 3.2 m at spring tide. Runoff is largely controlled by the Santa Clara dam located 60 km upstream.

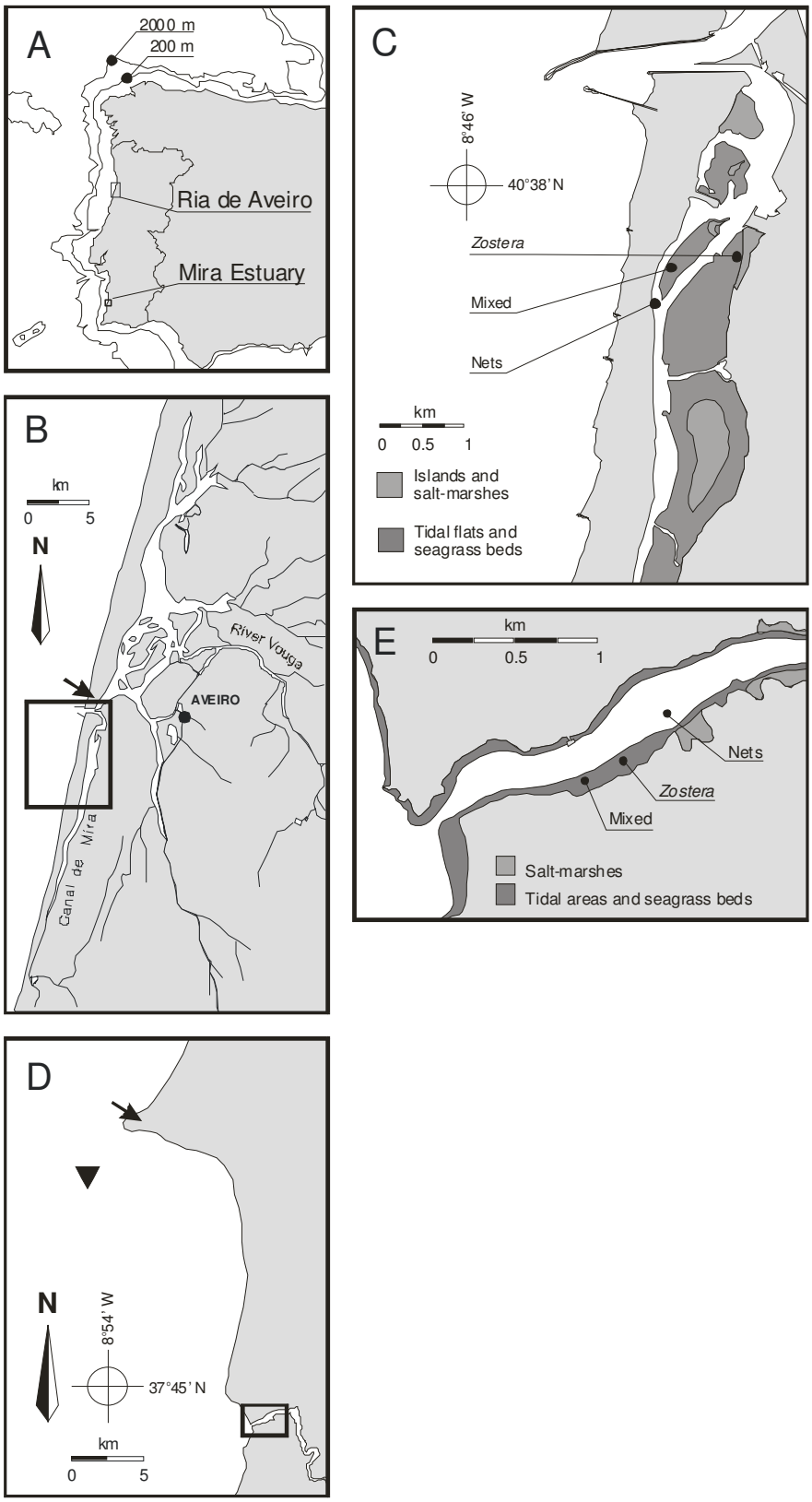

Figure 2.1. Sampling locations. (A) Iberian Peninsula; (B, C) Ria de Aveiro; (D, E) Mira Estuary. (C) and (E) show sampling sites. Arrows in (B) and (D): weather stations. Triangle in (D): hydrological buoy at Sines.

The Mira River has a limited and strongly seasonal freshwater flow, and since the dam was built it tends to accumulate sediments of marine origin in the lower reaches. The estuary waters are mixed during spring tides, with turbulence homogenizing the water column, and during neap tides stratification occurs (Andrade et al. 1991, Blanton et al. 2000). Fringing salt marshes occur as far as 20 km upstream. In the lower 8 km extensive seagrass meadows occur, subtidally *Zostera marina* and intertidally *Z. noltii*. The delay of the tide at the sampling sites relative to the inlet is similar to that observed in the Canal de Mira, Ria de Aveiro (Andrade et al. 1991).

Field sampling

The basic sampling strategy consisted of the acquisition of daily data on supply of megalopae, settlement of megalopae, wind stress, tidal range, SSL and sea surface temperature (SST) for periods of several months during the reproductive season of *Carcinus maenas*, at both locations. The sampling period lasted from 1 April to 25 July 2002 at the Ria de Aveiro and from 22 March to 6 July 2002 at the Mira Estuary, although not all data series within each location had the same length.

Supply of megalopae was measured with the use of passive plankton nets, which were specifically designed for sampling only during the flood tide. The nets had a length of 2 m, a rectangular opening of 0.40 × 0.25 m and a 500 µm mesh. Inside the net a funnel of 0.40 m length was attached to the opening frame. This funnel was also made of 500 µm mesh and had a circular posterior opening 0.17 m in diameter. Two of these nets were used at 1 site at each location (Fig. 2.1C, D), one floating just beneath the surface and the other placed above the bottom. Both nets were attached to tight stainless steel frames that could be placed above the bottom, suspended from a floating pier or made to float with the use of small buoys. Whether deployed over the bottom or near the surface, the opening of the nets always faced the inlet of the estuary. This was accomplished by securing the frames upstream and downstream with anchors, which were attached to the anterior and posterior ends of the frames, respectively. The attachment of the nets to the frames was done so that the

nets would freely filter the water during flood. During ebb both the interior funnel and the exterior net wall collapse, preventing the collected material from escaping. The nets were deployed during diurnal low water and remained in place for about 25 h until the next diurnal low water, when the contents were recovered. When low water occurred close to sunset, the nets were removed during the morning low tide of the second day, having been deployed for 37.5 h. The ability of the net design to prevent the loss during ebb of the plankton collected during flood was tested in an experiment at the Ria de Aveiro between 25 October and 23 November 2001. During this period, 5 sampling assays were conducted, each lasting for 25 h and starting at low water. In each assay 2 nets were set beneath the surface, parallel to each other, with one of the nets continually immersed for 25 h periods, while the other was immersed only during the 2 floods within each 25 h period. The plankton was separated into several taxonomic groups and counted (Table 2.1). A paired-sample Student's t-test (Sokal and Rohlf 1995) was applied to each taxonomic group. The difference between the nets was not significant for all taxa ($p > 0.05$), indicating that the net that fished continuously for 25 h periods retained the material collected during flood. In fact, the net that was continually immersed collected 15% more individuals than the other net. All analyses were conducted on number of megalopae collected daily by the nets, without correcting by filtered volume. The main reason for doing this is that during a large extent of the neap tides the speed of tidal currents is below the working range of most flowmeters. Therefore, attempts to standardise megalopae numbers by volume would be subject to a large error. Standardisation by volume would hardly affect the results, because differences in supply ranged from 0 (or almost 0) ind. d–1 during neap tides, to several tens 25 (Ria de Aveiro) or hundreds (Mira Estuary) ind. d–1 during spring tides, while differences in tidal currents between neap and spring tides are on the order of 1:2 at most. Moreover, standardisation would not affect the phasing of the supply peaks relative to tidal range, because they occurred around maximum amplitude tides.

Table 2.1. Average number of individuals (±1 SE) of several taxa collected by 2 passive plankton nets deployed for periods of 25 h and 2 consecutive floods. t-tests performed for paired samples.

Taxon	Continuous	Flood only	df	t	p
Copepods	62.6 ± 12.6	58.2 ± 9.1	4	0.405	>0.50
Isopods	129.4 ± 64.7	76.2 ± 8.5	4	1.769	>0.10
Amphipods	209.8 ± 50.1	258.6 ± 21.6	4	-2.532	>0.05
Mysids	630.6 ± 227.4	963.8 ± 28.5	4	-1.115	>0.25
Decapod larvae	306.6 ± 131.5	254.4 ± 16.3	4	0.344	>0.50
Appendicularians	104.2 ± 29.8	15.4 ± 4.4	4	2.738	>0.05
Chaetognaths	36.6 ± 24.9	54.6 ± 5.3	4	-0.753	>0.25
Fish larvae	20.8 ± 9.0	15.2 ± 3.9	4	0.857	>0.25
Total	1500.6 ± 346.8	1696.4 ± 45.3	4	-0.464	>0.50

Megalopae settlement was measured with the use of artificial collectors made with 'hoghair' filter material. Artificial collectors of this kind have been extensively used in studies of crab megalopae recruitment in coastal systems (e.g. Metcalf et al. 1995, van Montfrans et al. 1995, Paula et al. 2001). In the present case the collectors were not of the floating type as originally developed (Metcalf et al. 1995), but rather deployed over the bottom of intertidal flats. This methodology was used successfully at the Ria de Aveiro for the description of recruitment patterns of *Carcinus maenas* (Almeida and Queiroga 2003) and allows the separation of supply (as recorded from the density in the plankton measured with the plankton nets) from settlement. Two sampling sites were selected at each location: 1 area with soft mud densely covered with the eelgrass *Zostera noltii*, and a mixed area (Fig. 2.1C, E). The mixed area at the Ria de Aveiro was characterised by a muddy sand sediment with shells and sparse cover of *Enteremorpha* sp. and *Gracilaria* sp. At the Mira Estuary the mixed area also had muddy sand sediment, but with the presence of scattered boulders and a sparse cover of *Z. noltii*. In the Ria de Aveiro, 5 collectors with dimensions of 0.50 × 0.40 m were randomly deployed every day at each site, lying at distances of 2 to 4 m from each other, during the diurnal low tide, and fixed to the bottom with wire stakes. For logistic reasons one of the sampling

sites of the Mira Estuary had to be discontinued 67-d after the start of the experiment. Since numbers of megalopae collected at the *Z. noltii* site were much lower than at the mixed site, sampling proceeded at the mixed site. Moreover, only 4 replicates were used daily at each site in the Mira Estuary. Similarly to the plankton nets, the collectors were also deployed during the diurnal low water. In the next diurnal low tide they were removed, replaced by new collectors and taken to the laboratory in plastic bags. Crab megalopae and juveniles were recovered by immersing the collectors in fresh water, rinsing them with freshwater jets through a 0.5 mm sieve and sorting out the animals from the debris.

Hourly measurements of wind intensity and direction were obtained from a meteorological mast installed at S. Jacinto, in the case of the Ria de Aveiro, and from the Sines harbor weather station, for the Mira Estuary (Fig. 2.1B, D). The mast at S. Jacinto was situated 2.5 km to the northeast of the Ria de Aveiro inlet, on the sand bar that separates the northern part of the estuary from the sea. Sines harbor lies 22 km NNW of the Mira Estuary. The wind data were treated as follows. First, the hourly wind stress was calculated, for both east-west (T_u) and north–south (T_v) components, through the equation (Jorge da Silva 1992):

$$(T_u, T_v) = -\rho_a C |\vec{v}| (u, v)$$

where ρ_a is air density (1.12 kg m–3), C is a constant drag coefficient (0.012), v is wind-velocity vector, u is the east–west component of the wind (positive indicates easterly wind) and v is the north–south component (positive indicates northerly wind). Since the Portuguese west coast has an approximate north-south orientation, T_u and T_v correspond to cross-shore and alongshore stresses, respectively. Then the average daily wind stress for each component of the wind was computed.

Sea level data were obtained from the Aveiro and Sines harbor tide gauges, which are located at the inlet channel of the Ria de Aveiro, and on a hydrological buoy 5 km from Sines, respectively. Daily tidal range was computed as the difference between the average of the 2 daily maximum heights and the average of the 2 daily minimum heights. Daily SSL was calculated by running a

13-h moving average over the hourly sea level values in order to remove the tidal signal, followed by the calculation of the average of the filtered values for each day. Daily SST recorded at Leixões, 60 km north of the Ria de Aveiro, by the weather station of the Portuguese Instituto de Meteorologia, and by the hydrological buoy off Sines, were used in the study. The SST anomaly was calculated as the difference between the temperature at the coast and the temperature at an Azores station. SST anomaly at the coast relative to an offshore station not affected by upwelling has been used as an upwelling index.

For simplification, all references to wind and hydrological variables will be made as Ria de Aveiro or Mira Estuary.

Statistical analysis

In order to identify the nature of the mechanisms that control supply of *Carcinus maenas* megalopae it is necessary to identify the delay between tidal or wind forcing and the subsequent response of the coastal ocean and of larval supply. This was done using cross-correlation and least squares regression applied to series where wind or tidal range preceded hydrographical variables and supply, at lags ≤ 5 d (it is not possible to interpret meaningfully situations in which wind or tides precede the response of hydrographic variables or supply of larvae by >5 d). These lags are indicated throughout this paper by negative numbers. Spectral analysis and autocorrelation were used to identify the periodicity of the time series and the dependence of the data.

Upwelling theory predicts that an increase of the equatorward wind stress will cause a drop of the SSL and a decrease in SST at the coast. In order to investigate these effects and to determine whether the upwelling mechanism was operating during the study period, cross-correlations (Chatfield 1996) between the alongshore component of wind stress and SSL, SST, and SST anomaly were computed for time lags of 0 to –5 d. All data series were moderately to strongly autocorrelated at a lag of –1 d, as indicated by their partial autocorrelation function. This autocorrelation could inflate the cross-correlation values. In order to remove the autocorrelation all series were differenced before calculating the cross-correlations, using a time lag of 1 d.

Spectral analyses (Chatfield 1996) were also applied to the alongshore wind stress, in order to detect any periodicity of the wind, using the Kolmogorov-Smirnov d statistic to assess significance.

Cross-correlations were used to investigate the relationship between tidal range and abundance of megalopae in the nets and on the collectors, as well as to compare captures of megalopae in the nets and on the collectors, within and across locations. Again, because of dependence of the data at a –1 d lag, crosscorrelations were computed over the differenced series. Student's t-test was used to assess differences in the intensity of settlement on the collectors between the mixed and *Zostera noltii* sampling sites (Sokal and Rohlf 1995). Estimates of precision of the mean are reported as SE throughout the text.

Visual inspection of the data on abundance of megalopae in the nets indicated a very clear periodicity related to the tide amplitude cycle (see Figs. 2.5A and 2.6A). This was confirmed by spectral and cross-correlation analysis. Spectral analysis applied to the series of abundance of megalopae showed peaks in the periodograms at 14.6 and 14.0-d, and cross-correlation analysis indicated that correlation between abundance in the plankton nets and tidal range was highest at time lags of 0 and of –14 to –15 d for both locations. These observations suggested that tide amplitude was the primary factor controlling supply of megalopae to the estuaries. Therefore, in order to investigate the effect of wind on supply of megalopae the effect of tide amplitude had to be taken into account. This was accomplished by fitting a least squares regression model to the data on supply of megalopae, using, as the independent variables, wind and a set of sine and cosine functions accounting for the semilunar cycle of tidal range. Descriptions of the use of regression methods for the analysis of biological rhythms, which are usually referred to as periodic regression, can be found in Batschelet (1979) and deBruyn and Meeuwig (2001). In our case we used the property that any periodic function with a period equal to an entire number can be described by a linear combination of sine and cosine functions with periods equal to submultiples of the period of the main cycle (Wei 1990), and adjusted the model:

$$Meg_d = a_0 + \sum_{i=1}^{7} a_i \cos\left(\frac{2\pi i d}{14}\right) + \sum_{i=1}^{6} b_i \sin\left(\frac{2\pi i d}{14}\right) + c_i \tau_d + e_d$$

where Meg_d is the average daily abundance of megalopae in the 2 plankton nets (i.e. supply); d is the day of the year; τ_d is the average daily alongshore wind stress; a_0 to a_7, b_1 to b_6 and c_1 are regression coefficients; e_d is the random error term and i is an integer dummy variable that generates the periodic components (i runs from 1 to 7 in the case of cosine functions, and from 1 to 6 in the case of sine functions, since the sine of multiples of π is null). We chose 14 d as the approximation of the period of the semilunar cycle instead of the more commonly used value of 15 d, because the spectral analysis had shown strong components at periods of 14 and 14.6 d in the supply data. Variables that were not significant were removed, stepwise, from the analysis and a reduced model was fitted with the remaining variables.

We decided to use regression instead of ARIMA (auto-regressive integrated moving average) and Transfer Function models (Chatfield 1996, Brockwell and Davis 2002), because linear regression is a robust and flexible tool for discovering and modeling relationships among variables, which is our main goal in this work. It provides estimators for the coefficients that are optimal, as well as good estimates for their SDs, allowing accurate statistical tests on the significance of the variables in study. Furthermore, the coefficients and terms in the regression model have a clear biological and physical meaning, which is not the case in ARIMA.

Only the alongshore wind stress was used in the analysis because of upwelling theory and because crossshore wind stress was always positive during the period of study (except for days 78 to 90 at Mira Estuary) and showed small amplitude fluctuations (see Figs. 2.2A and 2.3A). The wind stress effect on circulation of the coastal ocean and settlement may occur with a delay of several days. In addition, this effect may not depend uniquely on the wind that was blowing in a particular day, but on the cumulative effect of wind events over several days. In order to test the effect of wind episodes spanning > 1 d, cumulative wind stress vectors were constructed by summing wind stresses over time windows of 2, 3 and 4 consecutive days, backwards from each day, for each

of the 2 locations. Cumulative wind stress vectors for > 4 d were not calculated, because previous studies (Fiúza 1984), and the spectral analysis of the wind time series reported in the present study (see Fig. 2.4), indicated strong periodicities of 6 to 9 d. Separate models were applied using lags of 0 to –5 d, in order to test for delayed effects of wind.

At both locations, for all combinations of wind stress vectors and lags between wind stress and supply, the residuals produced by the adjustment of the regression models presented an autocorrelation structure that followed a first order autoregressive process. This may result in incorrect significance values being assigned to the statistical tests (see Fuller 1976). However, under these circumstances, the least squares estimators of the coefficients keep their optimality properties, although their variances have to be recalculated taking into account the correlation structure of the residuals. For a first order autoregressive process this may be done applying the transformation $x_d - \varphi x_{d-1}$ to all the independent variates x, where φ represents the estimated autoregressive coefficient. Then, the usual formula for the variance/covariance matrix of the least squares estimators can be used, where the variance of the residuals is replaced by the variance of the white noise sequence associated with the autoregressive error term (Alpuim and El-Shaarawi 2008).

Because the overall level of supply, wind stress, and tidal range did not appear to consistently increase or decrease with time, trends were not removed. Supply of crab larvae to coastal habitats seems to be inherently ‘spiky’ (e.g. van Montfrans et al. 1995, Paula et al. 2001), with a few days with very high values interspersed with several days with zero or very low values. Moreover, visual inspection of the supply series did not detect higher variances associated with higher supply, indicating the absence of multiplicative effects (Chatfield 1996). Therefore, we decided not to take logarithms of the data in order to preserve their natural variability. A sensitivity test of the regression model (not shown) indicated a poorer fit to the log-transformed observations and a lower significance of the coefficients, but the time lags with the highest significant fits were the same.

Results

NNW winds dominated during the study period at both locations, as evidenced by wind stresses with a negative alongshore component and a positive cross-shore component of lesser magnitude on most of the days (Figs. 2.2A and 2.3A), but events of weak to moderate southwesterly winds were also observed. The most important wind events occurred from Days 70 to 77 at Mira Estuary, prior to the beginning of biological sampling, when peak alongshore wind stress reached 0.15 Pa, and from days 131 to 137 and 140 to 144 at Ria de Aveiro, with peak values of 0.04 Pa.

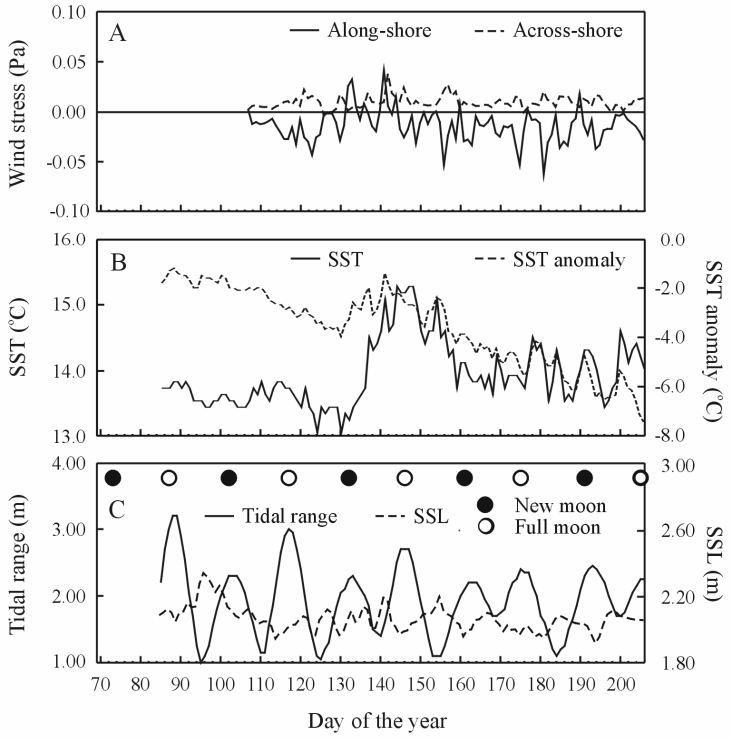

Figure 2.2. Ria de Aveiro. Average daily values of (A) wind stress, (B) sea surface temperature (SST) and SST anomaly and (C) tidal range and subtidal sea level (SSL).

The 2 last wind events were of shorter duration and reached lower stress values at Mira Estuary. Wind stress values during northerly winds were considerable higher at Mira Estuary (maximum value of –0.15 Pa) than at Ria de Aveiro (maximum value of –0.06 Pa). This may be related to the fact that the velocity sensor at Mira Estuary was located at a height of 50 m above ground, while the sensor at Ria de Aveiro was at 10 m.

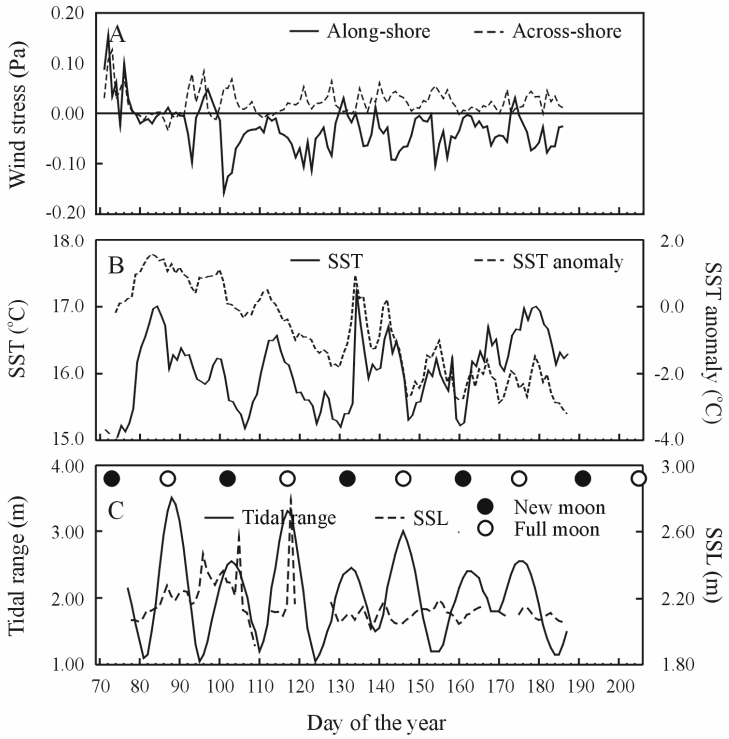

Figure 2.3. Mira Estuary. Average daily values of (A) wind stress, (B) sea surface temperature (SST) and SST anomaly and (C) tidal range and subtidal sea level (SSL).

Results of the spectral analysis of the alongshore component of wind stress revealed several spectral density peaks at periods of 2 to 9 d at both locations (Fig. 2.4, Ria de Aveiro: Kolmogorov-Smirnov d = 0.1980, n = 102, $p < 0.01$; Mira Estuary: Kolmogorov-Smirnov d = 0.2092, n = 126, $p < 0.001$). At Ria de Aveiro, 2 major peaks were detected at periods of 3.3 and 6.1 d, while 4 lesser peaks at Mira Estuary were detected at periods of 2.5, 3.6, 4.5 and 8.7 d. Significant cross-correlation values of alongshore wind stress with SSL, SST, and SST anomaly were always positive at both locations, indicating that an increase in northerly winds caused a decrease of SSL and SST at the coast (Table 2.2). However, the magnitude and timing of the response of the coastal ocean to the wind events differed between the 2 locations. At the Ria de Aveiro, a larger and quicker response was observed, with highly significant ($p < 0.001$) values for SSL at a 0 d lag, and for SST and SST anomaly at a lag of –1 d. At the Mira Estuary, SSL and SST anomaly were not associated with wind stress at any lag, but a significant ($p < 0.05$) association with SST was observed at a lag of –5 d.

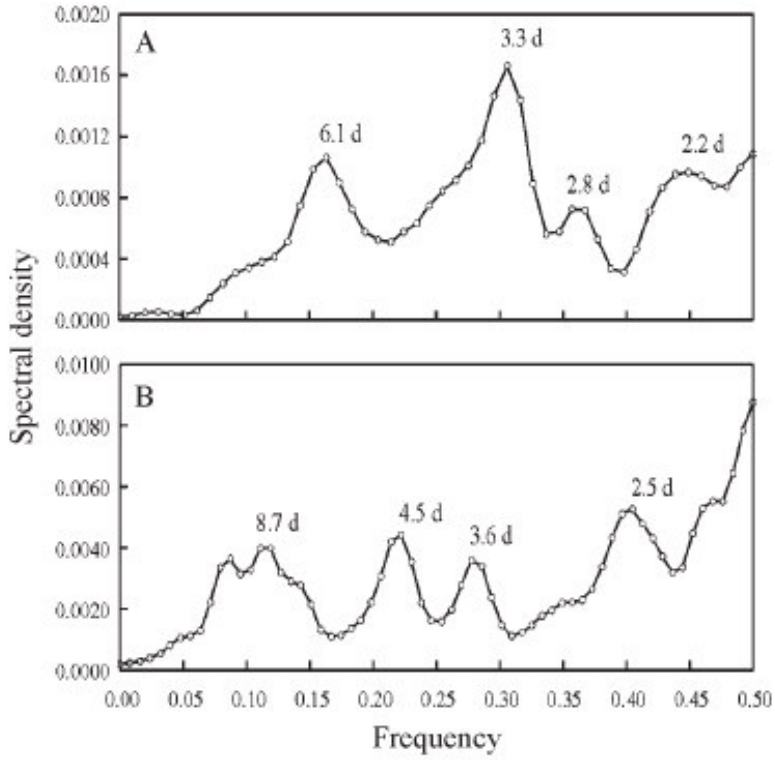

Figure 2.4. Spectral analysis of alongshore component of wind stress measured at (A) S. Jacinto, Ria de Aveiro, and (B) Sines, Mira Estuary. Numbers above peaks represent the period of oscillation.

Table 2.2. Cross-correlation analysis between alongshore component of wind stress and subtidal sea level (SSL), sea surface temperature (SST), and SST anomaly, for Ria de Aveiro and Mira Estuary. Only significant correlations are reported. *p < 0.05; ***p < 0.001.

Location	Lag (d)	SSL	SST	SST anomaly
Ria de Aveiro	0	0.4805***	-	0.2087*
	-1	-	0.3467***	0.3775***
Mira Estuary	-5	-	0.2871*	-

Average daily captures of *Carcinus maenas* megalopae in the nets and on the collectors were much higher in the Mira Estuary than in the Ria de Aveiro (Table 2.3), being 18, 45 and 31 times higher in the nets and at the mixed and *Zostera noltii* sites, respectively. Supply of *C. maenas* megalopae to the estuaries, as measured by the number of megalopae collected daily by the passive nets, was clearly more intense around the full and new moons at both locations (Figs. 2.5A and 2.6A).

Table 2.3. *Carcinus maenas*. Mean daily capture of megalopae in nets (ind.net–1.d–1) and on collectors (ind. collector–1 d–1) at the mixed and *Zostera noltii* sites, for Ria de Aveiro and Mira Estuary; n: no. of observations.

Location	Site	n	Mean	SE
Ria de Aveiro	Nets	102	7.860	1.07
	Mixed	115	0.350	0.07
	Zostera	115	0.070	0.02
Mira Estuary	Nets	57	142.320	32.00
	Mixed	110	15.90	3.45
	Zostera	64	2.34	0.51

Spectral analysis of the supply series showed peaks in the periodogram at 14 and 14.6 d at the Ria de Aveiro and the Mira Estuary (Ria de Aveiro: Kolmogorov-Smirnov d = 0.5700, n = 102, $p < 0.001$; Mira Estuary: Kolmogorov-Smirnov d = 0.3639, n = 56, $p < 0.001$), and cross-correlograms between tidal range and number of megalopae in the nets showed positive peaks at lags around 0 and –15 d at the 2 sites (Fig. 2.7A, C), although values at the Mira Estuary were not significant at the 5% level. Captures of megalopae by the passive nets also differed across locations in that the surface and bottom nets collected similar numbers at the Ria de Aveiro (surface 7.8 ± 1.2, bottom 7.9 ± 1.1), whereas at the Mira Estuary the surface net collected more megalopae (surface 267 ± 65, bottom 19 ± 4). This is reflected in the larger SEs obtained at the Mira Estuary.

Table 2.4 reports the results of the application of the periodic regression models supply of *Carcinus maenas* megalopae on a set of sine and cosine functions describing the semilunar cycle and on wind stress, for those cases where wind stress was significant. In all cases the sine and cosine variable with a period of 14 d was significant, indicating a basic semilunar periodicity in the series of larval supply. In the case of the Ria de Aveiro the cosine variable with a period of 2.8 d was also significant in 1 case, indicating the presence of a cyclic component with a higher frequency.

Table 2.4. Results of periodic regression models of megalopae supply to Ria de Aveiro and Mira Estuary on alongshore wind stress and on sine and cosine functions describing the semilunar cycle of tidal range, for those cases where wind stress was significant. Window: time window over which accumulated wind stress values were calculated. Values in parentheses: period of sine and cosine functions. Only independent variables used in the reduced models are reported (see 'Results' for further explanation). *p*: Significance of regression coefficients ($p <$ specified value); r^2: modified coefficient of determination.

		Regression coefficients					***p***					
(d)	(d)	Intercept	Wind	Cos(14.00)	Sin(14.00)	Cos(2.80)	Intercept	Wind	Cos(14.00)	Sin(14.00)	Cos(2.80)	r^2
Ria de Aveiro												
0	1	9.661	155.368	4.790	4.930		0.0001	0.0004	0.0156	0.0117	0.622	
0	2	9.760	81.137	4.818	4.932	1.281	0.0001	0.0108	0.0135	0.0106	0.0291	0.655
Mira Estuary												
-1	1	256.013	2663.381		122.738		0.0001	0.0059		0.0264		0.369
-2	1	263.967	2870.178		130.998		0.0001	0.0028		0.0168		0.386
-1	2	299.110	1839.301		126.955		0.0001	0.0036		0.0196		0.430
-2	2	264.377	1450.748		134.338		0.0004	0.0261		0.0194		0.386
0	3	292.924	1184.013		120.566		0.0002	0.0158		0.0274		0.376
-1	3	301.172	1250.050		131.658		0.0002	0.0116		0.0178		0.418
0	4	304.492	959.110		125.442		0.0003	0.0187		0.0220		0.380

Because of the existence of a correlation structure in the residuals (reflecting the influence of megalopae supply of any one day on that of the next day) the usual way of measuring the fit of the model through the *r*2 statistic is not appropriate in this case. Instead, we used a modification of this statistic, *r**2, where the sum of squares of residuals was replaced by the sum of squares of the white noise sequence produced by the adjustment of an autoregressive process to the residuals. In this case, this sum of squares is a more realistic measure of the variability due to unexplained error. Overall fits, as measured by the *r**2 statistic, were better at the Ria de Aveiro than at the Mira Estuary. At the Ria de Aveiro a significant effect of the wind was detected only for lags of 0 d, while for the Mira Estuary significance of the wind was obtained with lags of 0 to –2 d. At both estuaries the highest fits were obtained for the cumulative wind stress series calculated over time windows of 2 d, indicating a possible influence of wind events spreading over 2 d. In all cases the wind stress coefficients were positive, i.e. southerly winds resulted in increased supply of megalopae to the estuaries. Cross-correlation analysis between larval supply at the Ria de Aveiro and the Mira Estuary did not give significant results at any lag (data not shown). Settlement in the collectors showed strong peaks during neap tides around quarter moons at the Mira Estuary, but clear patterns related to the semilunar cycle were not detected at the Ria de Aveiro (Figs. 2.5B, C and 2.6B, C).

Crosscorrelations analyses between tidal range and captures in the collectors for the mixed site (Fig. 2.7B, D), for which longer times series were available at both locations, showed a pattern for the Mira Estuary, with negative values at lags around 0 and –15 d; this contrasts with the pattern obtained for the plankton nets (Fig. 2.7C). There was no periodic pattern on collectors at the Ria de Aveiro. These results indicate that settlement was decoupled from supply within estuaries.

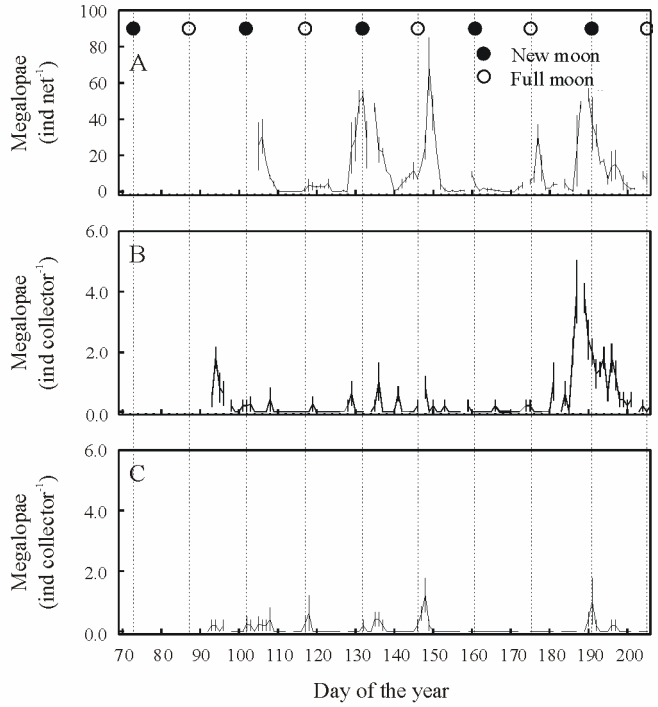

Figure 2.5. *Carcinus maenas*. Average daily number of megalopae collected by (A) passive nets, and collectors at (B) the mixed and (C) *Zostera noltii* sites, in Ria de Aveiro. Error bars: ±SE.

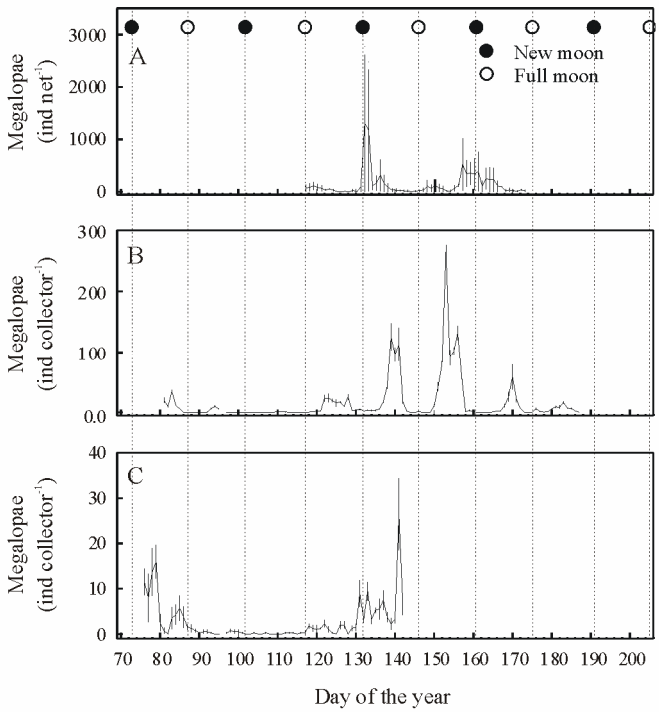

Figure 2.6. *Carcinus maenas*. Average daily number of megalopae collected by (A) passive nets, and collectors at (B) the mixed and (C) *Zostera noltii* sites, in Mira Estuary. Error bars: ±SE.

Daily captures of *Carcinus maenas* megalopae by the collectors (Figs. 2.5B, C and 2.6B, C, Table 2.3) at the mixed sites were significantly higher than at the *Zostera noltii* sites (Ria de Aveiro $t = 4.221$, $n = 115$, $p < 0.001$; Mira Estuary $t = 3.259$, $n = 62$, $p < 0.01$). Correlation between settlement in the mixed and in the *Z. noltii* sites was highly significant at the Mira Estuary ($r = 0.44$, $n = 64$, $p < 0.001$) and close to statistical significance at the Ria de Aveiro ($r = 0.17$, $n = 114$, $p < 0.07$) at a lag of 0 d. Therefore, settlement appears to be synchronized across sites within estuaries.

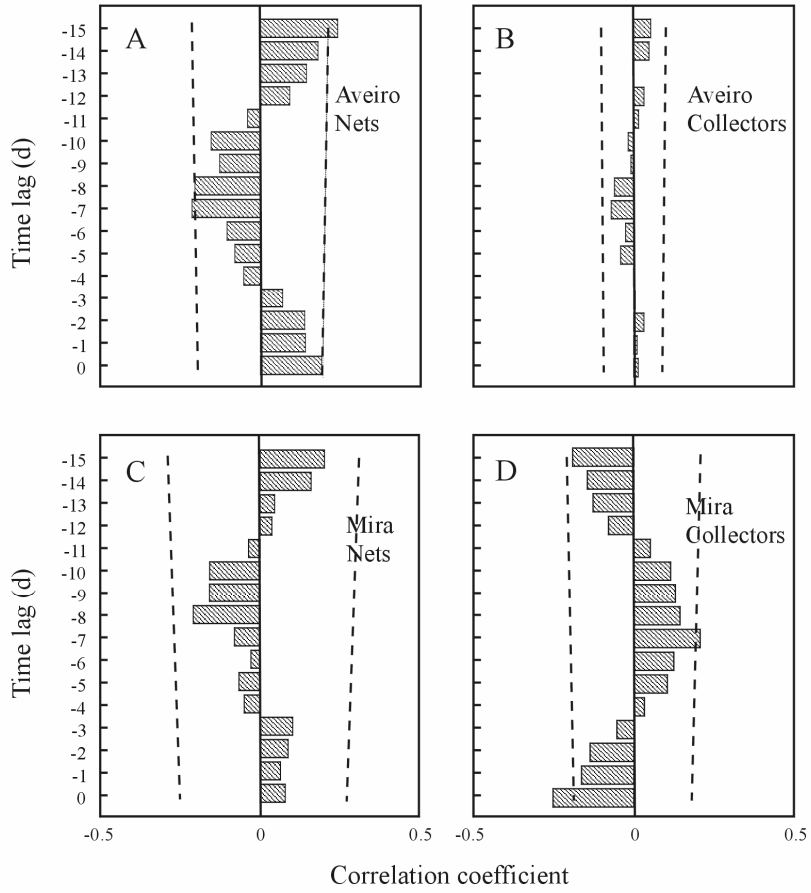

Figure 2.7. *Carcinus maenas*. Cross-correlograms between tidal range and number of megalopae collected by passive nets at (A) Ria de Aveiro and (C) Mira Estuary, and between tidal range and number of megalopae from collectors deployed at (B) mixed site at Ria de Aveiro and (D) Mira Estuary. Dashed line: approximate 95% CI.

Discussion

It is often difficult to identify causal relationships in the natural environment (Shrader-Frechette and McCoy 1993). In the present study such a problem results from the attempt to describe processes that occur on the shelf from observations made inside the estuaries; ideally, inferences on the mechanisms for cross-shelf transport of larvae should be based on observations made on the shelf, in order to avoid the confounding effect of changes in behavior and physical processes taking place as the larvae enter the estuaries (Shanks 1998). We attempted to minimize this problem by setting our sampling stations within 1 tidal excursion from the inlets of the estuaries, to reduce the possible effects of accumulation of larvae between the inlet and the sampling stations, and to maximize the link between larval abundance inside the estuaries and in the nearshore waters. A second shortcoming created by the lack of observations on larval abundance on the shelf is that a decrease in supply level need not mean that the appropriate physical setting for transport is not occurring, but rather that there are no larvae to be transported. Therefore, the predictive power of our models decreases and this could obscure the role of other variables. Nevertheless, the results reported in the present study support the 2 main hypotheses under test, i.e. that supply of megalopae to estuaries on the Portuguese west coast occurs with a semilunar periodicity and that supply levels are higher during relaxation of equatorward (upwelling-favorable) winds. Differences were found between the northern and southern locations regarding the response of the coastal ocean to wind forcing, overall supply levels, vertical distribution of megalopae in the water column, and site-specific settlement.

Wind-driven supply

Northwesterly winds dominated the study period, in agreement with the expected scenario. Maximum positive and significant correlation values of SSL, SST and SST anomaly with wind stress were observed at time lags of 0 and –1 d at the Ria de Aveiro (north coast), indicating a quick response of the coastal ocean to changes in the intensity of the alongshore wind. In the Mira Estuary (southern coast) variations of SSL and SST anomaly were uncorrelated with wind

stress, while SST was strongly correlated at a time lag of –5 d. The nature of the wind regime also differed between the 2 geographic locations, with 2 dominant periods of oscillation of 3 and 6 d in the north, and 4 less defined periodic components varying from 2 to 9 d in the south.

A positive effect of wind stress on supply of *Carcinus maenas* megalopae was detected at both locations, supporting the hypothesis that onshore convergence of the surface layer during these periods transports the larvae shoreward. However, slight differences between the northern and southern locations concerning the response of supply were observed. At the Ria de Aveiro the effect of the wind was significant only at a lag of 0 d, indicating a quick response of the transport mechanism that matched the delay between wind stress and changes in sea level and surface temperature. At the Mira Estuary a slower response was detected, reflected in time lags of 0 to –2 d for the most significant effect of wind stress on supply. These results suggest that the differences in the patterns of supply of megalopae between the northern and the southern locations are due to differences in the response of the coastal ocean to wind stress patterns in terms of the cross-shore components of circulation.

The influence of wind circulation on larval supply also depends on the vertical distribution of the larvae. Relaxation or reversal of upwelling-favorable winds will result in coastward advection of larvae provided they are retained in the upwelling front (Roughgarden et al. 1991) or remain in the surface Ekman layer, but offshore transport will occur if the larvae occupy the bottom Ekman layer (Blanton et al. 1995). Using a modeling approach, Marta-Almeida (2006, this volume) showed that nocturnal diel vertical migration of larvae spanning several 10s of meters, a widespread behaviour in decapod larvae (Queiroga and Blanton 2004), may enhance retention of larvae close to the coast under upwelling conditions. The interpretation of the sign of the association and of the lags between wind events and supply depends on the position occupied by the larvae in the water column, as well as on the distance larvae lie from the coast and the rates of advection during downwelling. Rates of coastward advection of 5 to 10 km d–1 have been estimated for the Portuguese coast following

relaxation of northerly winds (Fiúza 1984). If the larvae are concentrated in the inner shelf as a result of vertical migration behaviour, a very short delay could result, explaining the time lags of 0 to –2 d obtained in the present study.

The relationship between upwelling circulation and larval supply may be more complex and involve more than cross-shore transport, because of strong alongshore currents that may distribute the larvae regionally and interact with coastal topography (Wing et al. 1995). It is possible that inter-regional differences - and, for that matter, also intra-regional patterns - detected in the present study may be explained by variation of the alongshore circulation and by larval availability.

Tide-driven supply

A 2 wk period of oscillation of supply of *Carcinus maenas* megalopae was found, with higher supply around spring tides. Other semilunar patterns of supply to estuarine populations have been detected in only 2 other crab species (*Callinectes sapidus*, van Montfrans et al. 1995; *Cancer magister*, Miller and Shanks 2004). Other cases of estuarine crabs where a similar pattern of megalopal supply was detected concern species assemblages from tropical or subtropical mangrove systems (Moser and Macintosh 2001, Paula et al. 2001) where resolution to species level was not possible due to unavailability of larval descriptions. Crosscorrelograms indicated a delay of 0 d between tidal range and supply of *C. maenas* megalopae in the present study. This is similar to the studies of Moser and Macintosh (2001) and Paula et al. (2001), but differs from the delays detected by Miller and Shanks (2004). Miller and Shanks (2004) found that maximum abundance of *C. magister* megalopae occurred 4 to 5 d after maximum amplitude of the tide, and they attributed this effect to a combination of onshore transport by internal waves and diel vertical migration on a background of tidal currents.

The immediate response of supply of *Carcinus maenas* megalopae to the amplitude of the tide in the present study is consistent with the immediate responses of megalopae to hydrostatic pressure, salinity and turbulence during selective tidal stream transport, which increase with increasing tidal range in

megalopae of estuarine species (De Vries et al. 1994, Tankersley et al. 1995 Welch and Forward 2001). These reactions can only be triggered if the megalopae are already in nearshore waters or inside the estuary, resulting in an immediate change in larval concentration in the lower estuary. It has been argued that, since supply of crab megalopae into estuaries tends to occur during night flood tides, the number of flood hours during darkness could influence the level of supply (Christy and Morgan 1998), resulting in a different phasing of supply relative to the spring/neap cycle. In the present case, since co-occurrence of darkness and flood currents is optimized at spring tides during the part of the year when the study took place (Queiroga and Blanton 2004), such an effect may be responsible for the intensification of supply peaks, but not for their displacement relative to the tide amplitude cycle.

On the basis of the observed lags between tidal range and supply, the hypothses that internal tidal bore and internal wave mechanisms are implicated in the onshore transport of megalopae cannot be entirely rejected, although they appear to be less strongly supported. If internal tidal bore transport should occur, it would operate also on megalopae that are close to the shore, again resulting in an immediate supply. In a study conducted on the west coast of the USA, Pineda (1995) showed that internal tidal bores recur with a 2 wk periodicity, but that they do not tend to coincide with spring tides. The mechanism explaining this phase relationship is that, although the formation of internal waves is more probable during spring tides because of stronger currents, large amplitude tides tend to disrupt the thermocline, so that internal waves break far from the shore. In order for internal tidal bores to be implicated in the supply of *Carcinus maenas* megalopae to estuaries on the Portuguese coast, internal bores would have to coincide with maximum amplitude tides. A similar argument applies to the internal wave hypothesis. Additionally, assuming that the internal tidal wave mechanism is effective in transporting megalopae across the shelf, *C. maenas* megalopae are mostly not neustonic (Zeng and Naylor 1996b), which would result in a reduced rate of transport. A very particular timing of the occurrence of internal waves along the semilunar cycle would have to be assumed to account for the observed time lags.

Supply levels

Much higher levels of supply and settlement were detected at the Mira Estuary than at the Ria de Aveiro. This may be related to the distribution of large *Carcinus maenas* populations along the coast of Iberia, and to a general southward flow during the larval season. In the north, the Ria de Aveiro is the only estuarine system with the potential to harbour a large population of the species, whereas on the south coast the Tagus and Sado estuaries may contribute large numbers of larvae to shelf waters, and these larvae could be carried southward by the prevailing circulation during spring and summer. Supply was not correlated across regions at any lag. Since supply seems to be controlled by tidal range, which is synchronised along the Portuguese coast, the lack of correlation may be due to interregional differences in overall level of larval abundance, particularly if they are related to zones of retention over the shelf.
Abundances in the surface and bottom plankton nets were similar at the Ria de Aveiro, but more larvae were collected at the surface in the Mira Estuary. These differences may be related to higher turbulence levels that would force megalopae to swim more in the water column at the Mira Estuary (Welch et al. 1999). Interestingly, settlement was more intense at this estuary during neap tides, which may also be attributed to an effect of turbulence (see below).

Settlement

Settlement of *Carcinus maenas* megalopae on the collectors was synchronised across habitats within each estuary, but was higher at the mixed than at the *Zostera noltii* sites (Paula et al. 2006) conducted a comparison of settlement on collectors against megalopal density on the sediment at the Mira Estuary, for vegetated and unvegetated substrates. The study showed that density of megalopae on the substrate was higher in *Z. noltii* habitats than at unvegetated sites, but that captures on the collectors were higher at the unvegetated sites. The pattern was interpreted as a search for the physical protection provided by the collectors at the unvegetated sites, where refuges are less common, whereas the natural structural complexity of the *Z. noltii* habitat would provide such refuges. Therefore, the higher settlement on the

collectors at the mixed site observed in the present study may be an artifact of the sampling method influenced by differences in habitat complexity among sites.

Similarly to supply, settlement on the collectors was not correlated across geographic locations. Desynchronised settlement across locations would be a logical result of desynchronised supply. However, maximum settlement occurred around neap tides at the Mira Estuary, therefore being clearly decoupled from supply, which is highest around spring tides, while settlement was uncorrelated with supply at the Ria de Aveiro (although the correlogram of Fig. 2.7C shows an indication of a weak synchronised rhythm). Several different causes could explain the observed pattern. One possible explanation assumes the influence of turbulence levels (Welch 1991), which may differ between estuaries or sites. Higher turbulence levels in the Mira Estuary, which are consistent with current velocities that are 10 to 40% higher at the Mira Estuary for tides of the same amplitude (Paula 1993, Queiroga 1995), could prevent the megalopae from settling during spring tides. The competence state of the megalopae may also delay settlement. Competence is defined as the ability of larval forms to respond to settlement stimuli (Crisp 1974). In crab megalopae competence depends on the physiological stage of the larvae (Forward et al. 2001). Several studies indicate a progression of the intermoult stage of crab megalopae as they approach settlement habitats (Metcalf and Lipcius 1992, Morgan et al. 1996, Zeng et al. 1997, Moksnes and Wennhagen 2001), and differences in intermoult stage can sometimes be found within estuaries (Lipcius et al. 1990). It is possible that the delay between supply and settlement in the Mira Estuary could be due to supply from a pool of megalopae in an earlier stage of development (Hasek and Rabalais 2001). Density-dependent secondary dispersal, such as that detected in juveniles of several benthic invertebrates (e.g. Turner et al. 1997, Reyns et al. 2004) is another possible factor. During spring tides at the Mira Estuary, a large number of settling megalopae may be offset by those leaving after settlement, because of crowding. In either case, this highlights that supply of larvae to estuarine systems may be decoupled from settlement, because of estuarine, site-specific, or larval characteristics.

The results obtained in the present study demonstrate a cyclic pattern of supply of *Carcinus maenas* megalopae to estuaries on the west coast of Portugal, related to the tide amplitude. The 0 d time lag observed between tide amplitude and supply suggests that selective tidal stream transport is the main process responsible for the supply of the larvae to the estuaries, although the internal wave and internal tidal bore mechanisms cannot be entirely rejected based on the observations. Selective tidal stream transport can only operate on larvae that have already been transported to nearshore waters by some other process. Supply to nearshore waters appears to be at least partly controlled by the dynamics of wind-driven circulation. The sampling methods that were used may be relevant for other studies of the effects of physical forcing on supply and settlement of estuarine invertebrates, because a combination of passive plankton nets and settlement bottom-deployed collectors enable the independent assessment of supply and settlement.

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Chapter 3

Use of artificial substrate collectors in estimating densities and growth rates in shore crab juveniles

Use of artificial substrate collectors in estimating densities and growth rates in shore crab juveniles

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ABSTRACT

Artificial substrate collectors made of hoghair filter have been used in studies of crab megalopae recruitment in numerous coastal systems all over the world. In a previous study, conducted to measure daily settlement of *Carcinus maenas* megalopae in Ria de Aveiro estuary, NW Portugal, we observed that bottom-deployed collectors captured high numbers of juvenile *C. maenas*. In this paper we describe several field experiments conducted to understand the possible uses of artificial substrate collectors in juvenile shore crabs studies. First we assessed the efficiency of the collectors in estimating crab juveniles densities according to habitat complexity. Our results demonstrate that the use of artificial collectors to measure densities must be carefully considered as juveniles appear to use of the collectors as refuges in the less complex habitat, while in the more complex habitat the crabs preferred to take shelter among the naturally-occurring seagrasses. It was also conducted a time series study using collectors deployed daily, that allowed the description of the different juvenile instar abundances over time and the estimation of juvenile growth rates. This is a very valuable outcome since obtaining this type of information is particularly difficult.

Keywords: *Carcinus maenas*; juveniles; artificial settlement collectors; natural densities; growth rates; seagrass; *Zostera noltii*;

Introduction

Recruitment, in the broadest sense, is the addition of new individuals to populations or to successive life-cycle stages within a population (Caley et al. 1996). Variation in recruitment is considered to be a key component of population dynamics (Caley et al. 1996, Roughgarden et al. 1988), since quantifying the earliest life-history stages can reveal patterns of adult population abundance (Eggleston and Armstrong 1995; Hines and Ruiz 1995). Nevertheless, larval supply, subsequent settlement and recruitment are difficult to measure due to problems of defining the number of larvae capable of settling at a particular place and time, and of counting these small and cryptic individuals (Caley et al. 1996). Therefore, developing techniques to determine abundance of advanced larvae or early juveniles, at relevant temporal and spatial scales, is a requisite for meaningful ecological research on post-settlement processes and, eventually, to assess fisheries stocks (Metcalf et al. 1995).

Artificial settlement substrates are collectors that can sample passively over a predetermined period of exposure and depth. Due to the thigmotactic behaviour exhibited by many decapod larvae, which cling to encountered objects, artificial collectors integrate natural abundances with larval behaviour. Moreover, this methodology allows the description of settlement densities in a temporal scale and for these reasons artificial settlement substrates have been used successfully in numerous coastal systems all over the world, to estimate decapod settlement patterns (Metcalf et al. 1995). It is the case of the western rock lobster *Panulirus longipes cygnus* (Jamieson and Philips 1993), the fiddler crabs *Uca pugnax* and *U. pugilator* (O'Connor 1993), the blue crab *Callinectes sapidus* (Goodrich et al. 1989, Morgan et al. 1996, van Montfrans et al. 1990, 1995), the dungeness crab *Cancer magister* (Eggleston and Armstrong 1995) or the shore crab *Carcinus maenas* (Almeida and Queiroga 2003, Amaral and Paula 2007, Moksnes and Wennhage 2001, Queiroga et al. 2006).

The shore crab *Carcinus maenas* is native to the Atlantic coast of Europe and northern Africa, from Norway to Mauritania. It is a decapod species

eurythermal and euryhaline, found in a variety of habitats, including protected rocky shores, cobble beaches, sandflats and tidal marshes. Its large ecological plasticity combined with its aggressive competitive and predatory behaviour, made it a successful global invader, with established reproductive populations on both the east and west coast of North America, South Africa, Patagonia, Australia and Tasmania (Carlton and Cohen 2003, Yamada et al. 2005). Its widespread distribution, abundance in coastal and estuarine waters and its ecological important role constitute major reasons for its frequent use in experiments. *C. maenas* holds a complex life history that includes four pelagic zoeal stages, which are exported to shelf waters where larval development takes place to a final benthic megalopal stage (Queiroga et al. 1994). Megalopa is the stage of the life cycle responsible for reinvasion of the estuary, or lagoonal nursery habitats, where undergo their development until the adult stage. It actively selects the settlement habitats (Hedvall et al. 1998), showing preferences for structured habitats, such as intertidal vegetated habitats (Moksnes 2002, Almeida and Queiroga 2003, Silva et al. 2006). After settlement, megalopae suffers metamorphosis into the first benthic juvenile instar, which is about 1.33 - 1.52 mm in carapace width. Similarly to megalopae, young juveniles inhabit preferably shallow structured nursery areas, where high densities of different size classes of recruits could create mutual interferences that result in density-dependent growth, cannibalism and emigration from refuge habitats (Moksnes et al. 1998, Moksnes 2002). When juveniles attain maturity they leave the intertidal area and disperse to deeper habitats (Thiel and Dernedde 1994, Naylor and Kennedy 2003).

In this paper we describe several field experiments conducted to understand the possible uses of artificial substrate collectors in juvenile shore crabs studies. We first assessed the efficiency of the collectors in capturing crab juveniles according to habitat complexity by comparing captures in the collectors with two other methods: visual detection and hand-collection, and sampling of the superficial layer of the sediment. Then collectors were used to estimate habitat preferences and densities of juveniles in several intertidal habitats. Finally, we conducted a time series study using collectors deployed

daily, that allowed the description of the different juvenile instar abundances over time and the estimation of juvenile growth rates.

Methods

Study area and sampling sites

The study was conducted during the spring and early summer of 2002 at the Ria de Aveiro, a estuary located on the northwest coast of Portugal (40º38'N; 8º46W). Ria de Aveiro is a coastal shallow lagoon, separated from the sea by a sand bar and it is classified, in terms of geomorphology, as a bar built estuary (Pritchard 1967). Tides in the Ria de Aveiro, which are the main factor influencing circulation within the estuary, are semi-diurnal, with a mean tidal range of 2.1 m at the inlet (Dias et al. 2003). Our experiments were restricted to Canal de Mira, one of the branches of the lagoon, which, by its features can be considered, itself, a small estuary. Canal de Mira evolves from the inlet towards the southeast, parallel to the coastline and has an approximate extension of 20 km, a maximum width of 1 km and an average depth of 3 m (Dias et al. 2003). It holds large intertidal mudflats that are suitable for the megalopae recruitment and used as a feeding ground for both juvenile and adult crabs (Almeida and Queiroga 2003). Experiments took place among the intertidal area of Canal de Mira, in different habitat types (Fig. 3.1): at typical seagrass beds, dominated by *Zostera noltii*, which is a habitat with high structural complexity (*Zostera* 1 and 2), at a less complex habitat constituted by muddy sediment with a sparse cover of algae and shells (Mixed 1 and 2) and at an unvegetated habitat (Sand 1 and 2). We also tested a subtidal habitat, but data obtained from this habitat could not be included in the analysis.

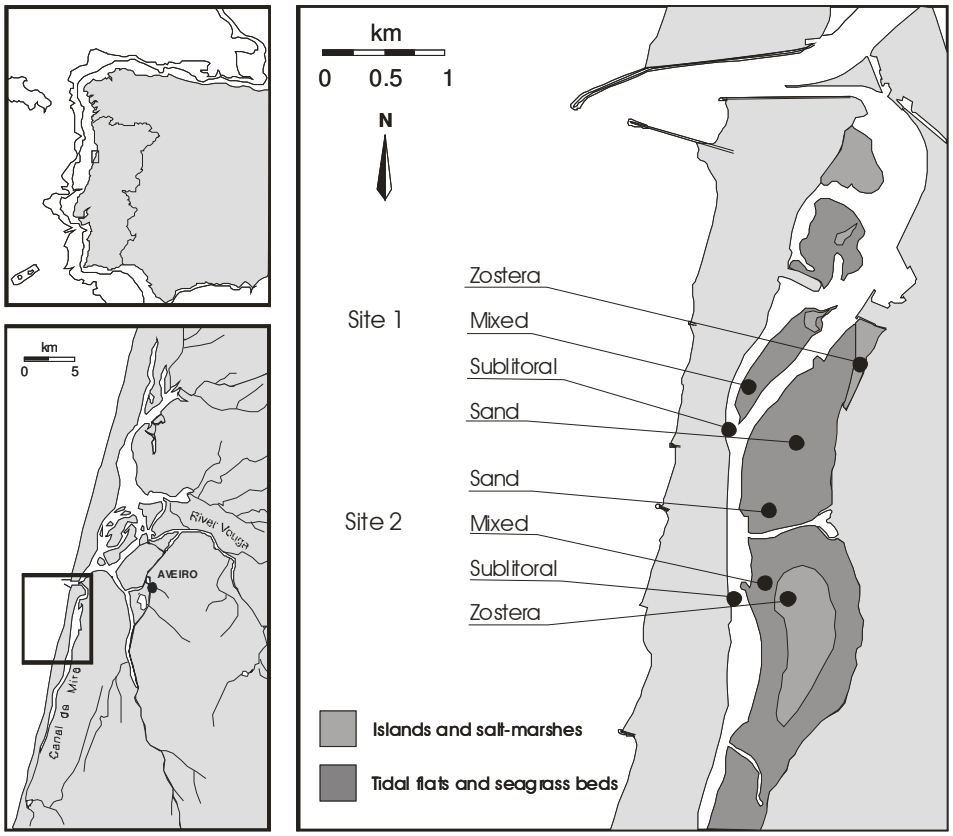

Figure 3.1. Location of Ria de Aveiro estuary and sampling sites.

Efficiency of artificial substrate collectors

The efficiency of artificial substrate collectors in estimating the densities of *Carcinus maenas* juveniles according to habitat complexity was estimated by comparing the captures of juveniles in the collectors with two other methods: visual detection and hand collection and sampling of the superficial layer of the sediment. The collectors that we used were made of 'hoghair' filter material and had dimensions of 50 x 40 cm. Collectors were randomly deployed over the bottom of the intertidal flats, during the diurnal low tide and remained there for about 25 hours, until the next diurnal low tide. By then they were replaced and transported to the laboratory where they were washed in freshwater. The rinsing water was filtered through a 0.5 mm sieve and all crab specimens were collected. The other methodology used consisted in visual detection and hand collection of all juveniles present in an area enclosed by a 50 x 50 cm quadrat. We also collected the superficial layer of sediment, down to a depth of 2 cm, enclosed by similar quadrats (these samples were formalized for later sorting).

We assume that sediment sampling accurately measure the density of juvenile crabs, regardless of habitat complexity. This experiment was conducted at habitats Mixed (mixed 1 and 2), *Zostera* (*Zostera* 1 and 2) and sand (sand 1 and 2; Fig. 3.1). At each site, collectors were replicated 4 or 5 times and both manual and sediment collections were replicated 10 times. Later, the habitat Sand was excluded from statistical treatments because very few juveniles were collected there. These trials were conducted in two different haphazardly chosen dates (14 June and 26 July, 2002).

Differences on shore crab juvenile densities were examined through a 3-way ANOVA model where Methodology (a fixed factor with 3 levels: artificial settlement collectors, visual detection and hand sampling and sediment collection), Habitat (a fixed factor with 2 levels: *Zostera* and mixed) and Site(Habitat) (a nested factor with 2 levels: sites 1 and 2) were the independent variables. Before running the ANOVA, a Cochrane test was used to evaluate assumption of homogeneity; since this was not achieved, data were logaritmized. The factors and interactions between factors that this ANOVA has shown to be significant were submitted to *a posteriori* SNK tests.

Densities and spatial distribution of juveniles

Artificial substrate collectors were also used to investigate habitat densities and preferences of shore crab juveniles. Experiments lasting six consecutive days where conducted at intertidal habitats, *Zostera noltii* meadows (*Zostera* 1 and 2) and mixed habitats (mixed 1 and 2) and also at a subtidal habitat (subtidal 1 and 2), where the collectors were deployed on the bottom of the channel (Fig. 3.1). Afterwards, these data could not be included in the analysis as the collectors trapped a large quantity of drifting algae (namely *Ulva* sp., *Enteremorpha* sp., and *Gracillaria* sp.) and it was not possible to distinguish if juveniles were effectively captured by the collectors or if they were carried by the algae. At each site, 4 collectors were deployed over the sediment during low tide, changed after 25 hours and rapidly transported to the laboratory, to recover crab specimens. These trials were replicated 3 times, in 3 different sampling periods (26-31 Mai, 9-14 June and 25-29 July, 2002). The carapace

width (CW, the width between the tips of the lateral spine) of all juveniles sampled was measured with calipers, to the nearest 0.1 mm or with an ocular micrometric, depending to the juvenile size. Based on their size, juveniles were grouped in the two classes: instars 1 and 2, which can be considered the younger recruits, and instars 3 to 7 that included older juveniles.

A 3-way ANOVA analyzed the influence of Instar (1-2 and 3-7), Habitat (*Zostera* and mixed) and Site(Habitat) (a nested factor with 2 levels: sites 1 and 2) on the mean density of juveniles captured per collector. Cochrane C tests indicated heterogenous variances. This condition could not be corrected by any of the usual data transformations and, therefore, the ANOVA was calculated on the ranks of the observations, as suggested by Zar (1996).

Assuming that sampling of the sediment (one of the methodologies applied before) is an unbiased method to estimate the densities of *C. maenas* juveniles, we used the data obtained previously to calculate correcting factors that must be applied to assess crab juveniles densities captured with artificial collectors. Original data was then corrected and the mean corrected density of juveniles was analyzed as a function of Instar, Habitat and Site(Habitat), with a similar 3-way ANOVA.

Temporal recruitment patterns and juveniles growth rates

Artificial substrate collectors were also used to assess temporal patterns of megalopae settlement and recruitment of juveniles. For this purpose, a time series experiment that was conducted from 3 April to 25 July, 2002 at sites *Zostera* 1 and mixed 1 (Fig. 3.1). The collectors (5 per site) and the methodology used to process them, have been described previously. Again, the carapace width of juveniles sampled was measured. Throughout the sampling period a total of 1,645 juveniles were collected, and of these, a random sub-sample of 115 was used for determination of stage and sex. An identification key adapted from Shen (1935) was used to define juvenile stages and sex according to variations in size and shape of endopodites and exopodites of the pereopods. Within this sub-sample the CW of juveniles ranged from 1.33 to 14.00 mm and seven juvenile stages were identified. Juveniles were then assigned to each

instar based on size. This analysis showed that juveniles up to and including instar 5 did not have overlapping sizes, instars 5 and 6 had an overlapping region of about 1 mm and instars 6 and 7 of 3 mm (Table 3.1; Fig. 3.2).

Table 3.1. *Carcinus maenas*. Minimum, maximum and average sizes (carapace width, CW in mm) and average increment of each instar of *Carcinus maenas* juveniles collected at Ria de Aveiro.

Instar	*Minimum CW (mm)*	*Maximum CW (mm)*	*Average CW (mm)*	*Average increment (mm)*
1	1,35	1,50	1,43	-
2	1,55	2,55	2,05	0,63
3	2,75	4,20	3,48	1,48
4	4,10	5,60	4,85	1,33
5	5,60	7,80	6,70	1,85
6	6,85	11,50	9,18	2,48
7	8,90	14,00	11,45	2,28

In order to estimate time lags between successive juvenile instars we used lagged cross-correlation analysis between time series of abundance of each instar, after smoothing with a 5 d moving average. Since the time series of abundance were significantly autocorrelated at a time lag of 1 d, the series were differentiated at this time lag. Because instar 5 and 6 showed only a modest overlap, juveniles were ascribed to each of these instars based on the average size of the overlapping range. Instars 6 and 7 were pooled together. We interpret the time lags between abundance patterns of successive instars given by this analysis as the duration of instars. The growth rates of each instar were then estimated by dividing the instar average size increment by the duration of the instar.

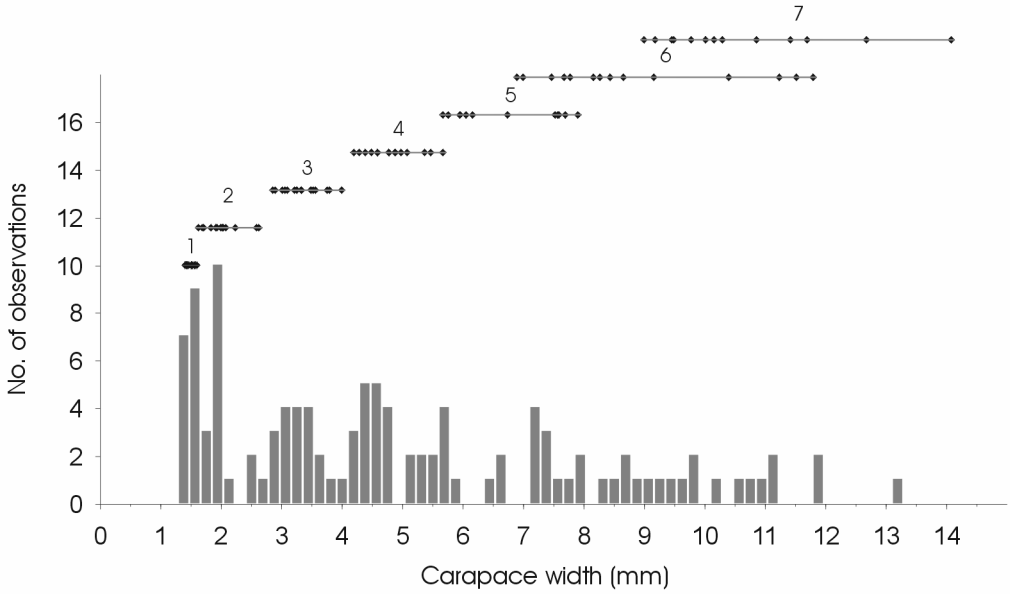

Figure 3.2. *Carcinus maenas*. Size frequency distribution for the juvenile population in the Ria de Aveiro. Above the bars it is indicated the length correspondent of each instar; each small point correspond to a single observation.

Results

Efficiency of artificial substrate collectors

Sampling of the sediment produced significantly lower estimates of densities of shore crab juveniles at mixed habitat than at *Zostera*. Manual collection of the juveniles produced estimates that followed the pattern of those measured by sampling the sediment; nevertheless, these results were significantly lower. On the contrary, densities assessed with the artificial collectors were significantly higher at them habitat (Table 3.2, Fig. 3.3).

Table 3.2. *Carcinus maenas*. Results of the 3-way ANOVA testing the effects of Methodology (artificial settlement collectors, visual detection and hand sampling and sediment collection), Habitat (*Zostera* and mixed) and Site(Habitat) (sites 1 and 2) on the log-number of juveniles.

	dF	*ms*	*F*	*p*	
Method	2	1,418	7,177	0,0000	***
Habitat	1	0,143	0,722	0,3974	
Site(Habitat)	2	0,444	2,245	0,1110	
Method x Habitat	2	4,023	20,362	0,0000	***
Method x Site(Habitat)	4	0,146	0,741	0,5665	
Residual	104	0,198			

*** $p < 0.001$

Densities and spatial distribution of juveniles

Although artificial collectors captured more juveniles at mixed habitat than at *Zostera* beds, at both habitats densities of juveniles from older stages are significantly higher than from younger stages (Table 3.3, Fig. 3.4). Besides the significant effect of Habitat and Instar on the densities of juveniles, the ANOVA also had shown a significant effect of the interaction between these two factors (Table 3.3). In fact, densities of the two juvenile instar classes are similar at *Zostera*, but at the mixed habitat, densities of stages 3-7 are significantly higher than stages 1-2.

After correcting the original densities data with the calibration factors calculated before, the results reversed: the preferred habitat for the juveniles i.e., where significantly more juveniles were captured was the *Zostera* meadows rather than the mixed habitats (Table 3.4, Fig. 3.5). Moreover, and unlike the original data, smaller juveniles were less abundant than older ones at both habitats. However, these differences were not statistically significant (Table 3.4). Therefore, at *Zostera* an average density of juveniles is 5.6 ind.m^{-2} while at the mixed habitat an average density of juveniles is 1.5 ind.m^{-2}.

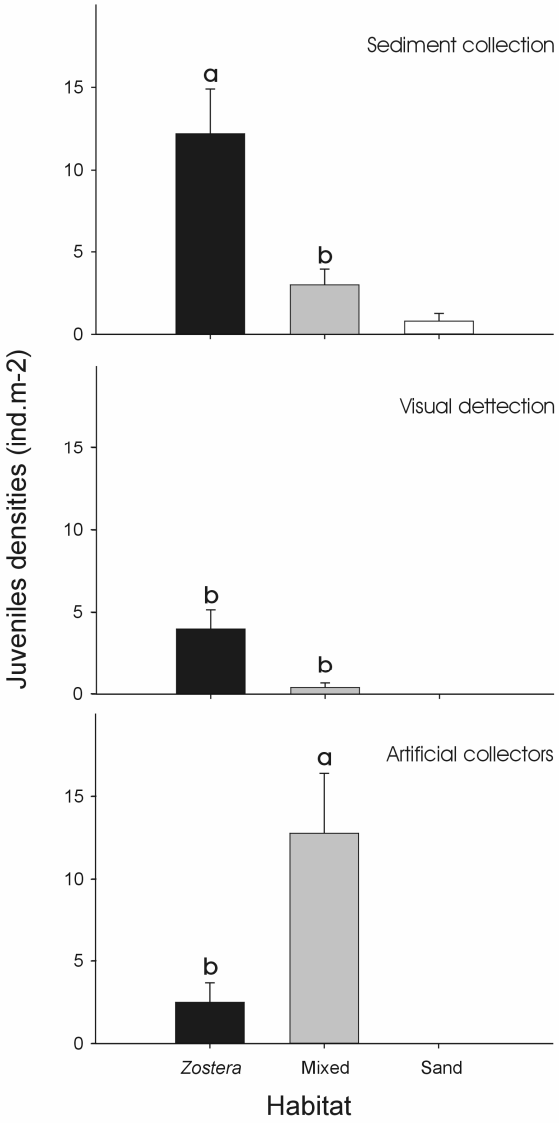

Figure 3.3. *Carcinus maenas*. Density of juveniles as a function of Methodology used and Habitat type. Bars and whiskers designate average values and respective standard errors, respectively. Distinct letters above bars indicate statistically differences among groups (SNK; $p<0.05$).

Table 3.3. *Carcinus maenas*. Results of 3-way ANOVA testing effect of Instar (stages 1-2 and stages 3-7), Habitat (*Zostera* and mixed) and Site(Habitat) (sites 1 and 2) on the ranked density of juveniles captured by the collectors.

	dF	*ms*	*F*	*p*	
Instar	1	482393	31,193	0,0000	***
Habitat	1	2352376	152,112	0,0000	***
Site(Habitat)	2	308511	19,949	0,0000	***
Instar x Habitat	1	302710	19,574	0,0000	***
Instar x Site(Habitat)	2	3984	0,258	0,1020	
Residual	581				

* p < 0.05; *** p < 0.0001

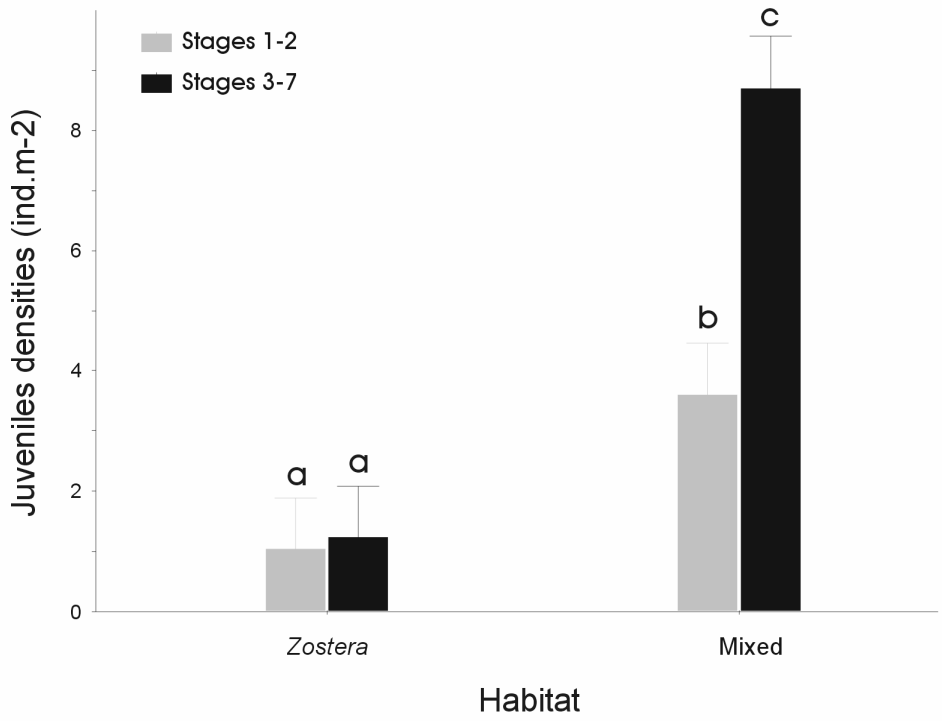

Figure 3.4. *Carcinus maenas*. Density of juveniles per collector (+SE) as a function of habitat. Distinct letters above bars indicate statistically differences among groups (SNK; p<0.05).

Table 3.4. *Carcinus maenas*. Results of 3-way ANOVA testing the effect of Instar (stages 1-2 and stages 3-7), Habitat (*Zostera* and mixed) and Site(Habitat) (sites 1 and 2) on the density of juveniles captured by the collectors, after the correction by the effect of Habitat.

	dF	*ms*	*F*	*p*	
Instar	1	180,716	2,142	0,1438	
Habitat	1	2178,509	25,823	0,0000	***
Site(Habitat)	2	564,526	6,692	0,0013	*
Instar x Habitat	1	2,239	0,0266	0,8706	
Instar x Site(Habitat)	2	4,046	0,048	0,9532	
Residual	581	84,363			

* p < 0.05; *** p < 0.0001

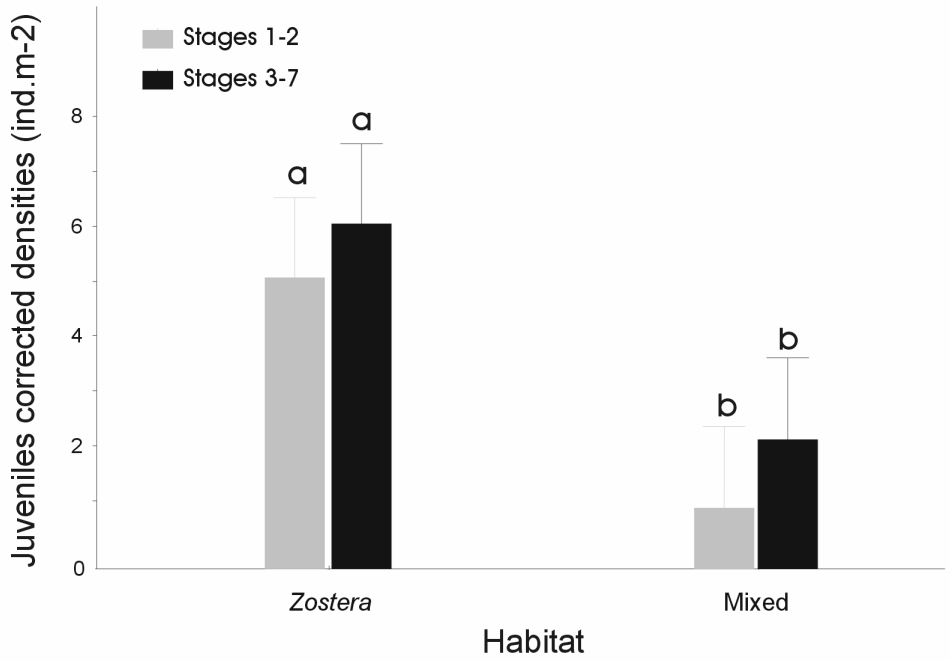

Figure 3.5. *Carcinus maenas*. Corrected density of juveniles (+SE) per collector as a function of habitat. Distinct letters above bars indicate statistically differences among groups (SNK; p<0.05).

Temporal recruitment patterns and juveniles growth rates

Size frequencies distribution, minimum, maximum and average sizes of the sub-sample of *Carcinus maenas* collected at Ria de Aveiro are shown in Table 3.1 and Fig. 3.2.

Densities of megalopae and different juvenile instars throughout the sampling period are illustrated in Fig. 3.6. Megalopae settlement was highly episodic with peaks that appear to be followed, almost immediately, by first instar juveniles density peaks. Similarly, these peaks are followed, over time by second instars density peaks. For instance, the megalopae settlement events that occurred during the first two weeks of April, can be matched to increases in abundance of instars 1, 2 and 3 that occurred, respectively, during the second and third weeks of April, mid to end of April, and third week of April to mid May. Similar patterns can also be recognized for the megalopae that settled during the third and fourth weeks of May and the second and third weeks of July. The presence of low numbers of fourth and later instars at the beginning of the time series suggest settlement episodes that took place earlier, in late winter.

In average, the duration of instars was about 1 wk, although it had ranged from 3 to 15 d (Table 3.5). Megalopae had the smaller period of duration (3 d) and juveniles at stage 4 had the longer period duration of 15 d. Growth rates, shown in Table 6, were variable according to crab stage: instar 2 grew ca. 0.09 mm per day, while instar 6 grew ca. 0.28 mm per day. Total time to reach instar 6 estimated by our study was 41 d, which lies between the 31 d and 69 d estimates by Klein-Breteller (1975) and Shen (1935), respectively (Table 3.6).

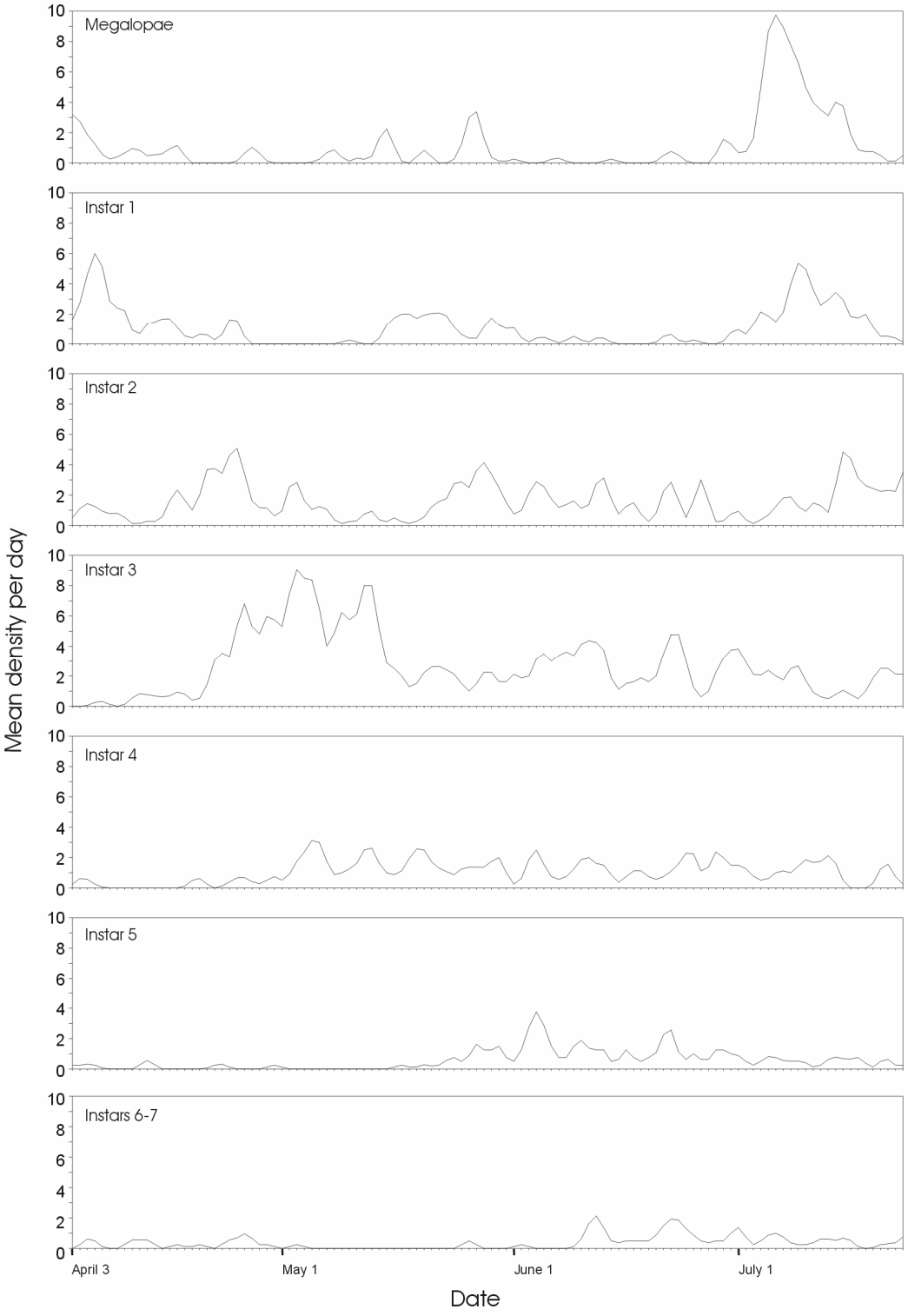

Figure 3.6. *Carcinus maenas*. Mean daily densities per collector of megalopae, instars 1, 2, 3, 4, 5 and 6-7.

Table 3.5. *Carcinus maenas*. Summary of significant results for cross-correlations and respective lags between megalopae and first instar, instars 1 and 2, instars 2 and 3, instars 3 and 4, instars 4 and 5 and instars 5 and 6-7.

	Cross-correlation	*p*	*Lag*
Megalopae and instar 1	0,5474	*	3 d
Instars 1 and 2	0,1788	ns	7 d
Instars 2 and 3	0,3161	*	8 d
Instars 3 and 4	0,2479	*	7 d
Instars 4 and 5	0,2243	*	15 d
Instars 5 and 6-7	0,2712	*	9 d

* $p < 0.05$

Table 3.6. *Carcinus maenas*. Average size (CW in mm), stage duration (days) and growth rates (mm per day) estimated for successive juvenile instars in these study. It is also presented similar data obtained from Klein-Breteller (1975b) and Shen (1935) studies.

	This study			**Klein-Breteller (1975b)**			**Shen (1935)**		
Instar	*Size*	*Stage duration*	*Growth rates*	*Size*	*Stage duration*	*Growth rates*	*Size*	*Stage duration*	*Growth rates*
1	1,43	7	-	1,50	4	-	1,90	7	-
2	2,05	8	0,09	2,10	6	0,15	2,36	12	0,07
3	3,48	7	0,18	2,89	7	0,13	3,01	14	0,05
4	4,85	15	0,19	3,98	6	0,16	3,75	18	0,05
5	6,70	9	0,12	5,57	8	0,27	4,95	18	0,07
6	9,18	-	0,28	7,84	-	0,28	6,21	-	0,07
7	11,45	-	-	10,33	-	-	8,32	-	-

Discussion

Efficiency of artificial collectors and spatial densities of shore crab juveniles

Young juveniles of motile benthic organisms such as crabs, lobsters or fish are often concentrated in structurally complex microhabitats such as mussel and seagrass beds, macroalgue and cobblestones (reviewed by Moksnes and Heck Jr. 2006). In particular, *Carcinus maenas* megalopae and first instar juveniles exhibit high plasticity in selecting habitats and exploring refuges among several heterogeneous substrates (Hedval et al. 1998, Almeida ad Queiroga 2003, Moksnes et al. 2003, Paula et al. 2006). Structured habitats are selected mainly because they provide shelter that prevents predation (Hedvall et al. 1998, Moksnes et al. 2003) and trap organic matter that increases growth (Perkins-Visser et al. 1996). Our results are in agreement with the referred literature: the three methodologies used in the first experiment, that assessed the efficiency of collectors, captured so few juveniles in bare sand, that we could not include data from this habitat in the statistical analysis.

Our results suggest that the differences in juveniles densities measured in the collectors and by sediment collection may result from the use of the collectors as refuges in the less complex habitat (mixed habitat), while in the more complex habitat (*Zostera* meadows) the crabs preferred to take shelter among the naturally-occurring seagrasses. In fact, the behaviour exhibited by shore crab juveniles is similar to the behaviour showed by shore crab megalopae, which settle preferentially on collectors deployed in unvegetated substrates, when compared to those deployed in seagrass meadows (Paula et al. 2006). Therefore, both studies suggest a strong interference between the collectors and the surrounding substrate, demonstrating that the use of artificial collectors to measure densities or settlement must be carefully considered. Paula et al. (2006) recommend caution in the use of artificial collectors for spatial variability studies; our experiments corroborate this proposition and also suggest that they are not the best methodology to estimate densities of juvenile shore crabs. One

way of solving this problem could be the calibration of data obtained with collectors with measurements obtained with a more reliable methodology.

It is also interesting to observe that corrected densities of shore crab juveniles estimated in structures habitats at Ria de Aveiro are extremely low (5,6 individuals per m^2), when compared to juvenile densities estimated at the Rio Mira estuary (146,5 individuals per m^2; Almeida et al. 2008) and at Sweden (68 individuals per m^2; Moksnes 2002).

Temporal recruitment patterns and juvenile growth rates

Although artificial substrate collectors are not a very accurate methodology to estimate abundances of juvenile *C. maenas* among different habitats, at least without carefully calibration, their use allowed us to determine natural growth rates of juveniles. Assuming that in a particular habitat the efficiency of collectors does not change over time, changes in abundance in the collectors should be proportional to changes in true abundance through time. Therefore, it should be possible to obtain a cost-effective measure of growth in natural conditions by inspecting the temporal differences between peaks of abundance of consecutive instars. This is a very valuable outcome since obtaining this type of information is particularly difficult. Field methods that could address this problem, like high frequency suction sampling or controlled cages could be a solution, but these methodologies are logistically complex to conduct or do not respect entirely the natural conditions. Due to these reasons, literature describing growth rates of *Carcinus maenas* juveniles is rare (Shen 1935 and Klein-Breteler 1975a, b). Moreover, those studies were based on laboratory experiments, where shore crab juveniles were raised from first crab stage and the growth of each individual was registered through time. Our study and the referred ones are not strictly comparable because our measure of growth was based in juveniles captured every day, instead of being based on the growth of the same individuals during time. In our field experiments crabs were subjected to natural conditions of temperature, salinity or the availability of food, i.e., the factors that most influence juvenile shore crabs growth (Klein-Breteler 1975a). But, because our results depend on the natural environmental conditions, they

should be more realistic and reliable than results obtained from laboratory studies.

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Chapter 4

Effect of crab size and habitat type on the locomotory activity of juvenile shore crabs, Carcinus maenas

Effect of crab size and habitat type on the locomotory activity of juvenile shore crabs, *Carcinus maenas*

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ABSTRACT

Post-settlement processes are presently a major focus in the study of the dynamics of marine populations and communities. Post-settlement movement of juveniles is an important but often ignored process, affecting local predator-prey and competitive interactions. We used benthic suction sampling and pitfall traps to examine density and locomotory activity of *Carcinus maenas* juveniles in different intertidal habitat types in the Rio Mira estuary, Portugal, to better understand intra-specific interactions in a system where density-dependent processes are known to regulate population dynamics. As expected, significantly higher densities of juvenile shore crabs were found from bare mud to densely vegetated habitats. At the time of sampling, small and intermediate stages together outnumbered by far larger juveniles. Conversely, larger crabs were much more frequent than smaller ones in traps. A locomotory index (LI), i.e. the ratio between crab catch in pitfall traps and their density within their moving range, is proposed as a measure of movement. LI analyses indicated that (1) movement is an order of magnitude higher in large than small juveniles and much higher in sparse than dense vegetation cover, (2) activity of small juveniles is mostly crepuscular, regardless of vegetation cover and (3) movement of large juveniles is very tenuous in dense *Zostera* patches, but very high in sparsely vegetated areas, mostly during the day and night. These results suggest that small juveniles are relatively protected under dense vegetation cover due to lower mobility of larger crabs, and evidence temporal segregation of activity windows between juvenile crabs of different size, which may be a key mechanism to reduce cannibalism and therefore increase carrying capacity of nursery habitats.

Keywords: *Carcinus maenas*; juveniles; habitat distribution; locomotory activity; Portugal.

Introduction

Many marine benthic organisms possess complex life histories with two or more developmental phases, in which demersal adult stages produce larvae that develop in the pelagic environment before recruiting to the benthos (Roughgarden et al. 1988, Ólafsson et al. 1994, Swearer et al. 2002). Recruitment success in these species is dependent on the supply of larvae to the appropriate settlement sites and on subsequent survival through the early benthic stages (Eggleston and Armstrong 1995, Shanks 1995, Wahle 2003). However, settlement and later dispersal of early juvenile stages can be critical periods in the life cycle of benthic organisms because of very high mortality due to predation, cannibalism or the absence of a suitable refuge habitat (reviewed by Hunt and Scheibling 1997).

Post-settlement movements of juveniles include dispersal and more oriented walking activities at different spatial scales. Foraging excursions and migrations are main patterns of movement and ultimately promote an optimized use of resources. Migration is an important process for local population dynamics that can mitigate crowding effects. The escape from risky areas can alter predator-prey and competition interactions, masking density-dependent relationships among early life stages (Moksnes 2002). Juvenile dispersal may also enhance the nursery capacity of estuarine systems by redistributing crabs from patches of high megalopal supply to areas where settlement is low (Etherington and Eggleston 2000, Etherington et al. 2003, Lipcius et al. 2007). Post-settlement dispersal may eventually determine recruitment patterns at broader scales, especially when juveniles can take advantage of pelagic transport, as showed for first stage swimming crabs of the genus *Callinectes*. Pelagic emigration of these juveniles takes place mostly during nocturnal flood tides and is more likely when intra-cohort benthic density is high (Reyns and Eggleston 2004). However, for most early crab stages, pelagic swimming is rare, and dispersal range is generally controlled by their walking activity. While emigration from crowded patches may reduce the impact of negative intraspecific interactions, as observed for the dungeness crab (Iribarne et al.

1994), different juvenile cohorts do extensively coexist under average natural densities in spite of strong cannibalistic regulation (Moksnes 2004). During most of the time, walking activity of small juvenile crabs in common nursery habitats is confined to much smaller scales, usually within tens of centimeters or unit meters, while crabs forage randomly. Such excursions configure short-scale dispersal, not migration, and may be timed in order to minimize predation risk.

Potential predators are perceived in many different ways by their prey, which may either suppress their activity at a large extent (Trussell et al. 2002), causing a decrease of growth rate (Trussell et al. 2003), or alternatively move to safer grounds (Heithaus et al. 2007), or change their temporal activity window to minimize the chance of coming across a predator (Grant and Brown 1998). In *Zostera* meadows along the Rio Mira Estuary, *Carcinus* juveniles are found in high densities and coexistence of different age-groups may well depend on optimal habitat exploitation over time. It may be predicted that small crabs shift the phase of their activity rhythm to avoid the risk of being cannibalized by larger individuals. Examining the diel variation of locomotory activity in these crabs may be a starting point to better understand patterns of habitat use in this important species.

There is no single specific methodology to measure post-settlement movements. Being a relatively recent concern, most contributions on this subject have adapted methodologies commonly used in other fields of research, such as mark-recapture techniques in commercially exploited decapod species (Wahle and Incze 1997, Smith et al. 2001, Stone and O'Clair 2001) and blue crab juveniles (Blackmon and Eggleston 2001, Etherington and Eggleston 2000, Etherington et al. 2003), a combination of trapping, acoustic telemetry and underwater video recording in the dungeness crab (Holsman et al. 2006), caging experiments in different estuarine and coastal brachyurans (Moksnes 2002, Moksnes and Wennhage 2001, Moksnes and Heck Jr. 2006, Iribarne et al. 1994), and pitfall traps, used in numerous studies to identify community structure and distribution of intertidal nekton and benthos (Moy and Levin 1991, Varnell and Havens 1995, Yozzo and Smith 1998, Able and Hagan 2000). This methodology was also used by Williams et al. (1985) to examine the activity patterns of New

Zealand mud crabs. None of these methods could be readily applied in our study system because we needed to measure locomotory activity for two juvenile categories in seagrass beds, as a measure of the incidence of short foraging excursions, rather than migration, as one would expect to record in otherwise bare bottoms.

In this study we first propose a novel method to measure walking activity of small juvenile shore crabs (stages 1 to 7), by coupling density estimates using benthic suction sampling and catch records by deploying pitfall traps. We then used the method to test the hypotheses that in natural nursery patches, where intraspecific interactions are intense, large and small *Carcinus* juveniles, corresponding to a predator/prey system, (1) exhibit different locomotory activity in sparse and dense vegetation cover, and (2) present mismatched diel activity patterns, in order to avoid negative intraspecific interactions, and therefore optimize the use of resources in a habitat where density-dependent processes presumably regulate population dynamics.

Methods

The green crab Carcinus maenas

The experiments described in this paper used the shore crab *Carcinus maenas* (L.) as model species. This is a dominant and highly motile crab in shallow coastal and estuarine habitats in Europe. The larval development of the green shore crab includes 4 pelagic zoeal stages and a megalopal stage (Ingle 1992), and lasts 4 to 6 weeks in shelf waters (see Queiroga et al.1994 for details). The competent megalopa actively select settlement sites (Hedvall et al. 1998), which are usually intertidal structurally complex habitats such as mussel beds, filamentous macroalgae or seagrasses (Thiel and Dernedde 1994, Moksnes 2002, Paula et al. 2006, Silva et al. 2006). Juvenile crabs remain in high densities in such habitats, which are known to provide adequate shelter from predation for these early stages (Moksnes et al. 1998, Moksnes 2002). Different

juvenile cohorts co-exist and cannibalism may greatly reduce the density of early recruits (Moksnes 2004).

Study area

The Mira estuary is a morphologically simple estuary, located on the southwest coast of Portugal (37°40'N, 8°40'W), running along a slate valley (Fig. 4.1). Tides are semi-diurnal, with ranges of about 1.0 m during neap tides and 3.2 m during spring tides. Tidal excursion in this estuary varies from 7.5 km during spring tides to 2.5 km during neap tides (Paula 1998), thus covering the estuarine sampled section almost continuously, and maintaining salinity always higher than 30 (Esteves et al. 2000). The vegetation cover (*Zostera noltii*) at the intertidal margins can be clearly separated in dense patches, close to 100% cover when shoots are laid over the sediment during low-tide periods (referred hereafter as 'dense *Zostera*'), and areas where plants are scattered and open mud make most part of cover (henceforth 'sparse *Zostera*'). The shallow subtidal zone adjacent to *Z. noltii* habitats is dominated by dense cover of the seagrass *Zostera marina*.

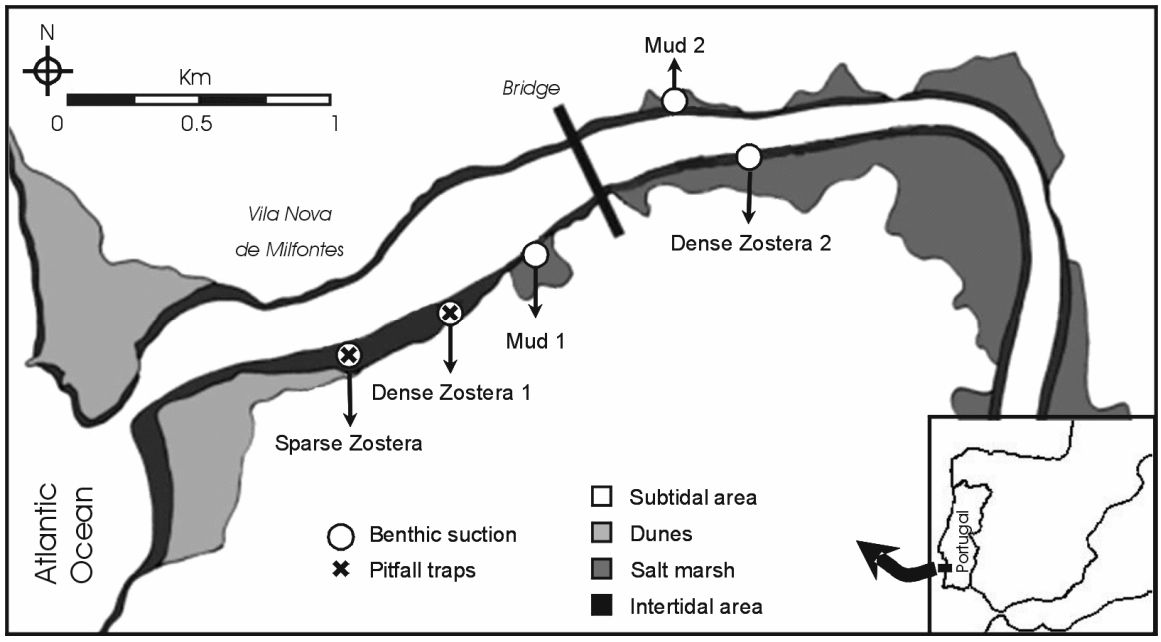

Figure 4.1. Map of the Rio Mira estuary showing the location of the study area and the sites where the different sampling methods were used.

Benthic suction sampling

Benthic suction sampling is a methodology used to assess densities of macrofauna in natural habitats. It has been widely used for more than a decade in several salt marshes, peat reefs and seagrass beds to sample juveniles of several decapods, including blue crabs *Callinectes sapidus* (Orth and van Montfrans 1987, Wilson et al. 1990, Etherington and Eggleston 2000, Heck Jr. et al. 2001), red king crabs *Paralithodes camtschaticus* (Loher and Armstrong 2000), shore crabs *Carcinus maenas* (Moksnes 2002) and American lobsters *Homarus americanus* (Able et al. 1988). In our study, a 1 m tall plastic cylinder, open at both ends, was used to enclose a bottom area of 0.110 m^{-2}. The cylinder was pressed into the sediment to prevent crab escape. The contents of the cylinder and the surface of the substrate were then emptied into a 500 µm mesh collecting bag, by using a Venturi-type suction device powered by a gasoline-driven centrifugal pump. Sampling efficiency trials were conducted in sparse *Zostera* and dense *Zostera* 1 sites (Fig. 4.1), but not at mud sites, where the substrate was completely bare and the suction sampling removed the entire superficial layer. At these two sites, five replicates were haphazardly chosen and sampled for one minute. The collecting bag was then replaced and, without removing the plastic cylinder, sampling was repeated for another minute. Again, the collecting bag was replaced and a last third sample of one minute was taken. Therefore, the total suction time for each of these preliminary samples was three minutes. The efficiency of sampling was determined after collecting the sediment enclosed in the cylinder and counting the number of remnant juvenile crabs. The results of these trials revealed a relatively high efficiency of the suction sampling method, but variable among sites. For the first two sampling minutes, approximately 86% of juvenile green crabs were captured in sparse *Zostera*, and approximately 70% for the dense *Zostera* 1 site.

The density of juvenile crabs was examined during the spring and early summer of 2003, which is the reproductive season of *Carcinus maenas* along the Portuguese coast. From April to July crab juveniles were sampled biweekly with a benthic suction sampler, over 9 sampling dates. Sampling took place at five intertidal sites (Fig. 4.1) throughout a substrate complexity gradient; from

unvegetated mudflat areas (mud 1 and mud 2), toward a sparse vegetation cover (sparse *Zostera* site), characterized by a muddy sand sediment with a thin cover of *Z. noltii* and finally to dense *Z. noltii* meadows (dense *Zostera* 1 and dense *Zostera* 2), which was the most complex substrate type. At each site, three replicate samples were taken randomly, consisting of materials obtained after a 2 min suction time. All samples were collected around high tide and, usually, all five sites were sampled on the same day. Samples were preserved in buffered 10% formalin for later analyses. In the laboratory, *C. maenas* crabs were counted and carapace width (CW, the maximum width between the tips of lateral spines) was recorded. Crabs were measured with a micrometric ocular.

C. maenas juveniles were separated into three different size classes according to Silva et al. (2006). The groups selected were very small crabs that included first to second stage juveniles (with a CW smaller than 2.5 mm), medium juveniles, incorporating third through fifth stages (from 2.5 to 7.5 mm CW) and large juveniles, from the sixth to the seventh stage (crabs larger than 7.5 mm CW). Juvenile crab densities were adjusted for sampling efficiency, i.e., 86% for sparse *Zostera* and 70% for dense *Zostera* 1. At mud sites we assumed that no correction factor was needed.

Differences in crab densities among the different sites were examined through separated 1-way ANOVAs, for each size class. As variances were found to be heterogenuos, data were square-root transformed. Replicates were deleted randomly in treatments to achieve balance (n = 23), because sampling was not always possible at all sites due to weather constraints. The Cochran's *C* statistic was used to test for homoscedasticity. Analyses were run even when variances were found heterogeneous, because the procedure is robust when sampling size is large (Underwood 1997). *A posteriori* multiple comparison tests were carried out using the Student-Newman-Keuls (SNK) procedure.

Pitfall trap experiments

Dispersal of *Carcinus maenas* juveniles was examined by using pitfall traps made from buckets. Pitfall traps have proved to be quite effective in catching *C. maenas* and have been used to examine the habitat use of juveniles within the

intertidal region of the Wadden Sea (Thiel and Dernedde 1994) or in the control and monitoring of adult green crab *Carcinus maenas* in the US, where this crab is considered to be an important invader species (Yamada et al. 2005). This methodology has also been used by Williams et al. (1985) to examine the activity patterns of New Zealand mud crabs. Our pitfall traps had 16 cm of height and 12 cm of diameter, and an aperture cut in the bottom that was screened with a mesh, allowing water to fill the trap during flood and preventing it from floating.

Pitfall traps were buried within sparse *Zostera* and dense *Zostera* 1 (Fig. 4.1) beds, the same sites used in the benthic suction sampling, and recovered during the following low tide. Special care was taken to maintain the borders of the trap just below ground level and the trap entrance as smooth as possible to maximize the catch and minimize avoidance. By doing so, we assumed that size-related efficiency of traps is negligible. No replication was provided within the sparse and dense conditions because there was only a single sparse patch over the study area. These experiments were not conducted within the mud sites because their density, as estimated by suction sampling, was very low there (Fig. 4.3). Sampling was periodically undertaken over 45 d, continuously during four 3 d periods, namely at the transition from spring to neap tides, when low water took place at 12:00 and 24:00, and at the transition from neap to spring tides, when it occurred at 06:00 and 18:00. Since locomotory activity in this species is maximum during periods of high-tide (Naylor 1958), low water capture in traps at 06:00, 12:00, 18:00 and 24:00 would reflect activity at night, dawn, day and dusk, respectively. All crabs retrieved from traps (n = 4) were counted and their carapace width was measured.

According to Moksnes et al. 1998 and Moksnes 2004, *Carcinus maenas* juveniles are highly cannibalistic. These studies have shown that both medium and large juveniles can consume high numbers of the smallest size class, and largest juveniles can also consume the medium size class. Therefore, we investigated cannibalism within pitfall traps in the lab. These experiments included small and medium juveniles as preys and medium and large juveniles as predators on small juveniles. We also examined predation by large juveniles on

medium size juveniles. Control experiments included only a single size of crabs inside pitfall traps. For each prey/predator combination, 5 preys and 3 predators were included in the pitfall trap and 4 replicates were taken. Time effect was assessed for 6 h and 12 h experiments. These times were chosen to approximate an intermediate (6 h) and maximum (12 h) periods of time that the crabs spent inside the traps during sampling. We included filamentous algae in the buckets to reproduce the conditions of the traps in the field.

Locomotory index

In order to quantify movement, records from pitfall experiments described above must be standardized by natural density estimates of juvenile crabs in the field, because dispersal can be density- or size-dependent. Therefore, a locomotory index was calculated for each size class and site as:

$$LI_s = \frac{C_i}{D_i}$$

Where LI_s, is the locomotory index for site *s*; C_i, is the number of captures of size class *i* and D_i, is the natural density of size class *i*. Density values used to estimate this index were obtained by benthic suction sampling experiments, concurrent with the pitfall traps trials on course.

The locomotory differences were examined for juvenile stages 1-5, because small and medium size classes had to be pooled to obtain a better record; and for large juveniles, stages 6-7, through separated 2-way ANOVA models, where the LI was considered the dependent variable, and examined according to Site (a fixed factor with two levels; sparse and dense *Zostera*) and Period (a fixed factor with four levels; night, dawn, day and dusk). The data obtained over the whole sampling period were pooled, rendering 48 replicate observations for each level combination. *A posterori* multiple comparison tests were carried using the SNK procedure.

Results

Spatial density patterns

The benthic suction sampling collected a total of 1,081 *Carcinus maenas* juveniles, ranging from 0.98 mm to 31.35 mm CW (Fig. 4.2). The size distribution of these juveniles was strongly right-skewed. The bulk of all recorded individuals consisted of medium juveniles between stages 3 and 5, and approximately 90% of sampled crabs were smaller than 10.00 mm. Small and medium juveniles, which comprised recent recruits, are apparently from a different cohort than juveniles in stages 6-7 (Fig. 4.2), which were less abundant, probably due to post-settlement mortality (Moksnes et al. 1998, Moksnes 2004).

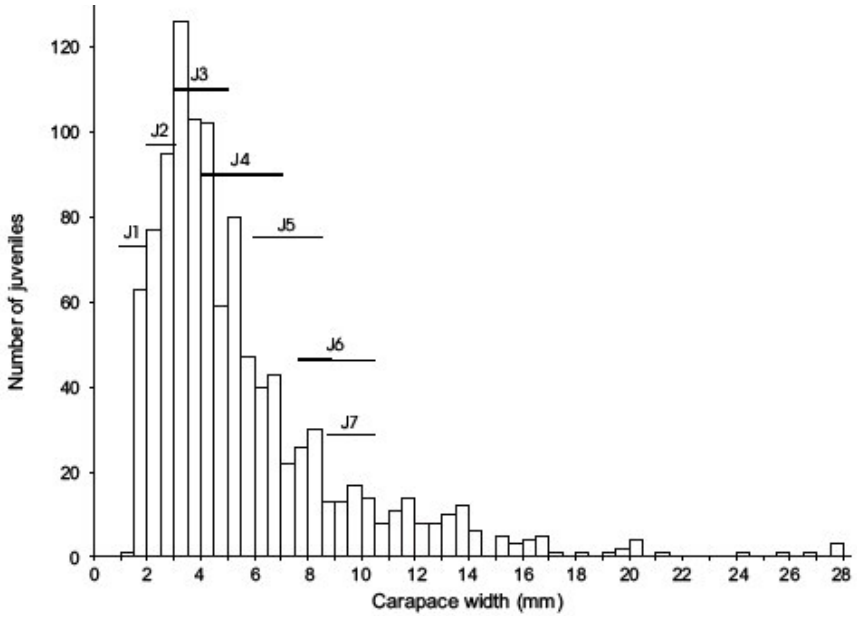

Figure 4.2. *Carcinus maenas*. Size frequency distribution of juveniles collected by benthic suction sampling. Size of stages according to Silva et al. (2006).

Density of all juvenile size classes tend to increase from muddy to vegetated sites. Within vegetated sites, density numbers of medium and large juveniles were higher in densely vegetated areas, significantly higher at dense *Zostera* 2 for large juveniles; but small individuals were more abundant in sparse *Zostera* (Table 4.1, Fig. 4.3).

Table 4.1. *Carcinus maenas*. Summary results of 1-way ANOVAs testing density differences among habitats for small (stages 1-2), medium (stages 3-5) and large (stages 6-7) juveniles. Data were square-root transformed and replicates were deleted randomly in treatments to achieve balance (n=23). C: Cochran's C statistic.

		small		medium		large	
	df	*F*	*p*	*F*	*p*	*F*	*p*
Crab density	4	7.3	***	14.9	***	12.4	***
Residual	110	*C*=0.398; *p*<0.01		*C*=0.406; *p*<0.01		*C*=0.326; *ns*	

*** $p < 0.001$

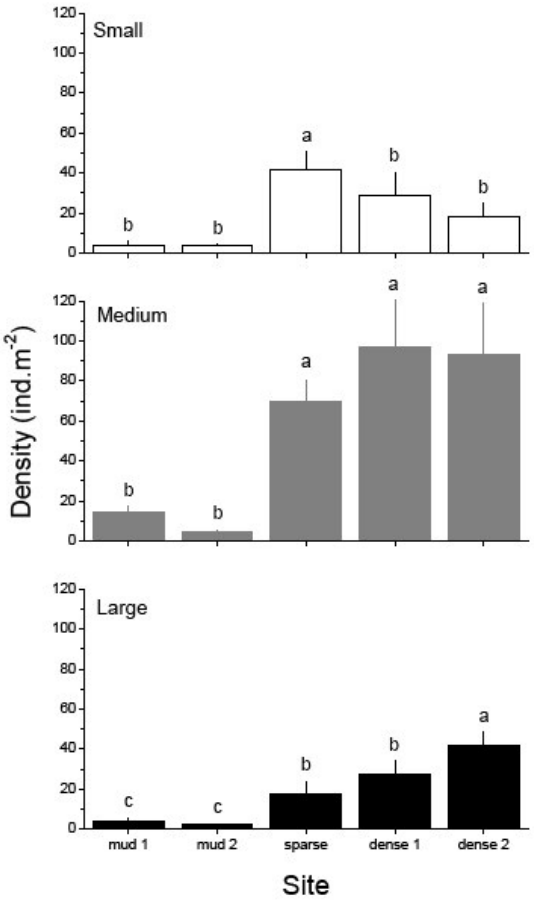

Figure 4.3. *Carcinus maenas*. Overall density of small, medium and large juveniles captured with the benthic suction sampler at the different sites. Bars and whiskers indicate average values and respective standard errors, respectively. Distinct letters above bars indicate statistically differences among groups (SNK; p<0.05).

Pitfall trap experiments

Large juveniles were the most captured crabs, with almost 80% of captures, while smaller juveniles were less frequent in buckets, representing only 1.6% of the catch. Pitfall traps located on sparse *Zostera* captured more juveniles, of all size classes, than traps deployed on dense *Zostera* 1 ($1.92 \leq t \leq 5.14$; $p < 0.05$ for all classes; Fig. 4.4).

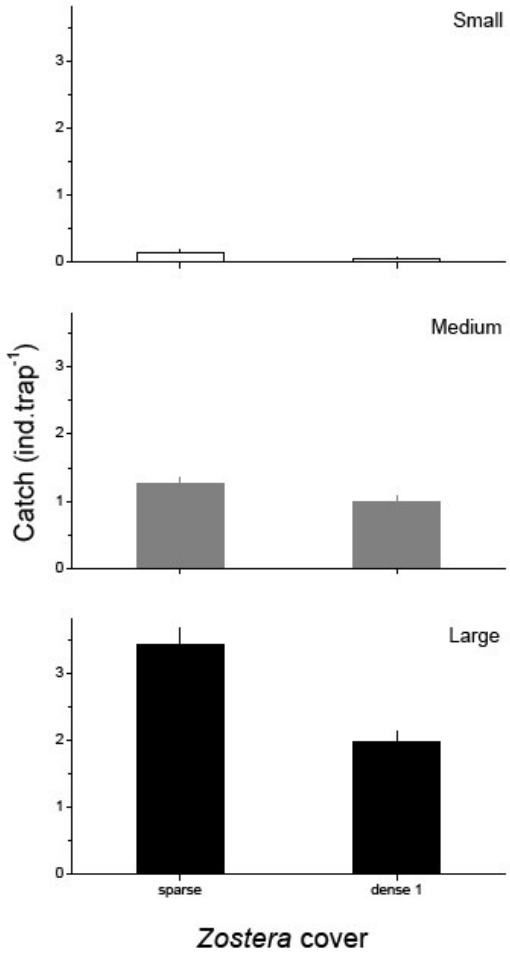

Figure 4.4. *Carcinus maenas*. Overall captures in pitfall traps for each juvenile size class, at sparse and dense Zostera 1 patches. Bars and whiskers as above. In all size categories, differences between dense and sparse vegetation cover are statistically significant (t-test; $p<0.05$).

The relative frequency of juvenile categories captured in pitfall traps at the sparse *Zostera* and dense *Zostera* 1 sites (Fig. 4.4) strongly contrasted with their density, as assessed with the benthic suction sampling (Fig. 4.3), showing

that captures in traps are not related to natural densities, but probably to different locomotory capacities between small and large juveniles. The possibility of biased catch estimates due to cannibalism in pits prior to trap replacement is rejected because no crab deaths were observed in laboratory trials.

Locomotory index

Large juveniles (Stages 6-7) are approximately an order of magnitude more active than small and medium juveniles (Fig. 4.5 and 4.6).

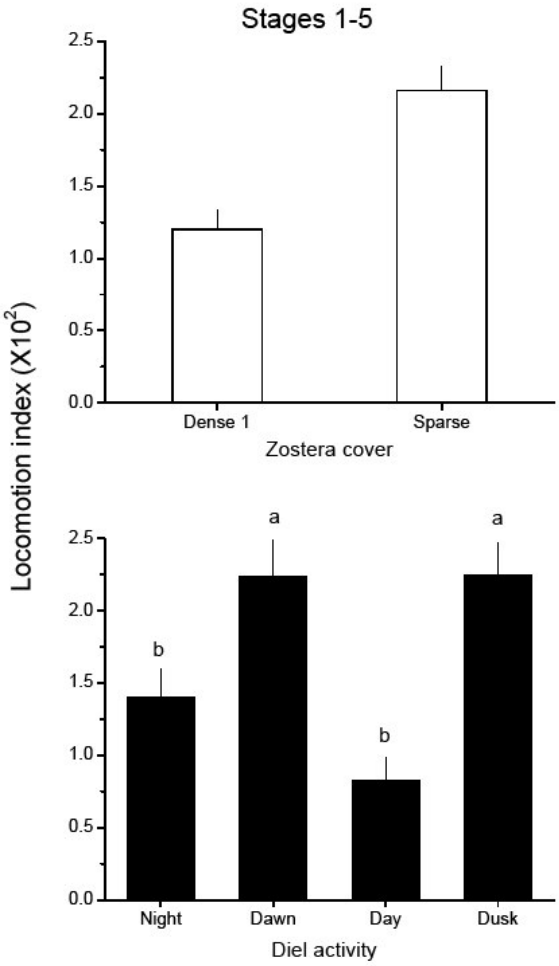

Figure 4.5. *Carcinus maenas*. Locomotory Index of juvenile stages 1-5 according to site (sparse and dense Zostera 1) and period (night, dawn, day and dusk). Lettering above bars as in figure 4.3 (SNK; $p<0.05$).

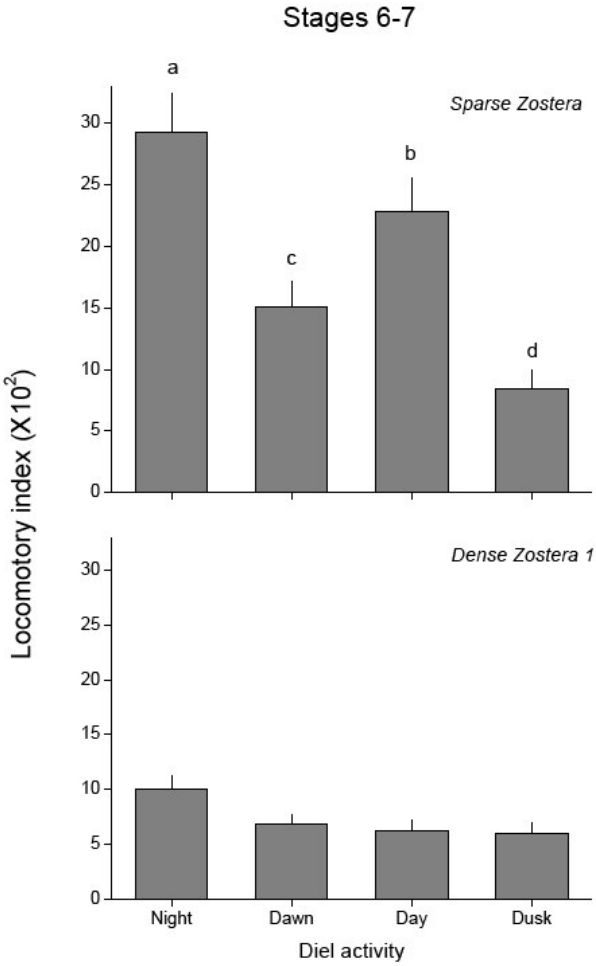

Figure 4.6. *Carcinus maenas*. Locomotory Index of juvenile stages 6-7 according to site (sparse and dense Zostera 1) and period (night, dawn, day and dusk). Lettering above bars as in figure 4.3 (SNK; $p<0.05$).

Both juvenile size classes were significantly more active at sparse *Zostera*, but, while the locomotory activity of 1-5 juvenile stages is mostly crepuscular, significantly higher during dawn and dusk, regardless the site (Table 4.2, Fig. 4.5), large juveniles are more active during day and night. However, for these juveniles, the diel activity is only significant at sparse *Zostera*, where the locomotory index is higher. At dense *Zostera* 1, activity was much reduced and no diel trends were observed.

Table 4.2. *Carcinus maenas*. Summary results of 2-way ANOVA models testing the effect of site (sparse and dense Zostera) and period (night, dawn, day and dusk) on the Locomotory Index of stages 1-5 and large 6-7 juveniles. C: Cochran's C statistic.

		Stages 1-5		Stages 6-7	
	df	*F*	*p*	*F*	*p*
Site	1	22.5	***	81.1	***
Period	3	11.7	***	16.7	***
Site X Period	3	2.2	ns	8.6	***
Residual	376				
		C=0.211; *p*<0.01		*C*=0.334; *p*<0.01	

*** $p < 0.001$

Discussion

Spatial density patterns

Seagrass beds have been usually considered the principal and optimal nursery grounds for many decapod species (blue crab *Callinectes sapidus*: Orth and van Montfrans 1987, Heck Jr. et al. 2001, Hovel and Lipcius 2002; brown shrimp *Farfantepenaeus aztecus*, white shrimp *Litopenaeus setiferus* and pink shrimp *Farfantepenaeus duorarum*: reviewed by Minello et al. 2003). These habitats have high structural complexity that offers protection from predation by fish and cannibalism (Moksnes et al. 1998, Orth and van Montfrans 2002). In addition, seagrass beds may enhance growth rates of juveniles through the provision of invertebrate prey (Perkins-Visser et al. 1996).

The results obtained in this study confirmed significantly higher densities of juvenile shore crabs in seagrass (on average 146.5 ind.m^{-2}) compared to mud habitats (on average 16.8 ind.m^{-2}). Our results are consistent with earlier studies that demonstrated the concentration of juvenile shore crabs in eelgrass beds,

and other structurally complex habitats, such as mussel beds and filamentous algae, rather than in open sand (Moksnes 2002). It is interesting, however, that densities were higher in sparse than dense *Zostera* for the smallest juveniles. It is possible that medium crabs may better prey on smaller recruits in dense *Zostera*, where larger crabs can not perform as well and therefore be less efficient to cannibalize intermediate juveniles. However, no attempts were made to test such a cascading effect. Alternatively, upstream settlement attenuation, as observed by Silva et al. (2006), may explain the higher density of small juveniles at sparse *Zostera*, closest to the estuary mouth. To our knowledge, these are the first quantitative data of habitat specific distribution of young juvenile shore crabs in Portuguese estuaries.

Locomotory activity

Similar to other studies (Thiel and Dernedde 1994, Yamada et al. 2005), pitfall traps produced very satisfactory results in the capture of *Carcinus maenas* juveniles. During our experiments, we collected over 1,446 individuals among the sites tested and were able to detect differences in the captures among different juvenile size classes. It is important to note that density estimates by suction sampling and catch in pitfall traps measure completely different features. Suction sampling is widely recognized as an adequate method to estimate density of soft-bottom invertebrates (see literature cited in methods). At the sampling time, we estimated much higher densities for small and medium crabs, altogether, compared to larger juveniles. Yet, this latter group was much more frequent in pitfall traps. Varnell and Havens (1995) concluded that pitfall trapping is a convenient and widely used method of collecting nekton from marsh surfaces, but it is not reliable to determine abundances of fishes and crabs. The results of our pitfall experiments support their argument: if catches were solely related to natural densities, we would expect a much larger frequency of smaller juveniles in traps. We conclude that such contrasting results largely reflect different abilities of these juvenile size classes to move over vegetated grounds. Size-related avoidance of pitfall traps seems very unlikely. Pitfall trapping thus appears to be a promising methodology to examine

post-settlement movements of juvenile crabs. Once pitfall catch records are standardized by natural density estimates, as assessed by efficient suction sampling, one may compare actual movement capability among different groups, in our case between small and large juvenile crabs. The locomotory index may thus render relatively unbiased measurements of movement. Cannibalism in traps did not occur, possibly because filamentous algae provide refuge for small juveniles and interactions among crabs are much reduced in such an unusual and likely stressing environment.

Locomotory activity of juvenile shore crabs was influenced by the phase of the day. However, the two size classes exhibited divergent diel movement patterns; while small juveniles were more active during dawn and dusk, large juveniles showed higher locomotion during the night and day. For larger juveniles these differences were only significant at sparse *Zostera*, where locomotion index is higher. In fact, dense seagrass may act as a physical barrier, inhibiting the movement of larger juveniles (Kneib 1995), therefore masking possible differences in locomotory ability during the daily periods at densely vegetated patches. This obvious temporal separation of locomotory activity of smaller and larger juveniles may be a very important mechanism to reduce cannibalism, particularly in sparse areas. In dense seagrass meadows cannibalism should be lower not only because the higher habitat complexity provides higher availability of refuges, as frequently suggested (e.g. Moksnes et al. 1998, Hovel and Lipcius 2001), but also because larger juveniles do not move so well. Hampered mobility in dense *Zostera* may thus prevent excessive cannibalism and therefore increase carrying capacity of seagrass meadows for *Carcinus* juvenile cohorts.

Several studies have already demonstrated that night-time dispersal is common in early life stages of many estuarine organisms as it reduces predation by visual predators (*Callinectes sapidus*: Blackmon and Eggleston 2001, Etherington et al. 2003). The results provided in this study partially support those observations - in fact larger juveniles were also more active at night than during the day - but the most striking pattern is the temporal segregation of activity windows of small and large juveniles. Most research on the effect of

predators or dominant conspecifics on their prey or subordinates, respectively, has been carried out in riverine and estuarine fish. Recent results strongly suggest that fish diel activity may be either suppressed in the presence of a predator chemical cue (Pettersson et al. 2001), or even reversed in subordinate, smaller fish, when dominant, larger conspecifics are removed (David et al. 2007). In this latter case, smaller individuals change to nocturnal activity, when drifting invertebrates, their main prey, are more abundant. In a predator-prey cannibalistic interaction involving juvenile cod age-groups, smaller fishes decreased nocturnal feeding seasonally, when larger individuals take over (Grant and Brown 1998).

Apart from clearly engaging a free-running tidal cycle, the green crab *Carcinus maenas* also exhibit a circadian component with peak activity at night (Naylor 1958). Naylor (1960) also verified that endogenous midnight activity is combined with lower activity peaks at noon. He proposed a multiple clock hypothesis, including a tidal clock (12.4 h) and another one of twice of this value, to account for the activity drift and the smaller nocturnal peaks of captive specimens held in constant conditions. At least in the field, larger juveniles do undertake this expected pattern, with a large nocturnal peak, and a second, less pronounced activity peak during the day (Fig. 4.6). Activity in smaller juveniles follows an opposite trend, with large crepuscular activity, possibly relying on their capacity to perceive larger conspecifics and avoid them. These results much resemble those obtained on talitrid amphipods (Kennedy et al. 2000, Naylor and Kennedy 2003). Therefore, the benefits of reducing the chances of coming across larger cannibalistic juveniles will surpass the disadvantage of missing nocturnal foraging where predation by other visual predators is lower. The confirmation of this mechanism needs, however, further laboratory analyses to test whether smaller recruits shift to an endogenous nocturnal activity pattern to match the predictable pattern of larger juveniles and adults.

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Chapter 5

Cannibalism among Carcinus maenas juveniles measured in situ

Cannibalism among *Carcinus maenas* juveniles measured *in situ*

Almeida M.J., Queiroga H.

Manuscript

ABSTRACT

Juvenile cannibalism may play an important role in population regulation of marine organisms with juvenile stages that aggregate in nursery habitats. Cannibalism in juvenile shore crabs *Carcinus maenas* were tested in an experiment using cages deployed at Ria de Aveiro (Portugal), with the intention of assess the influence of (1) prey and (2) predator sizes, and (3) the effect of presence of refuge on prey survival. The results demonstrate that cannibalism between juvenile shore crabs can cause high mortality at natural densities, particularly in smaller preys by medium and large size predators. Although not strictly significant at the 5% level, higher densities of *Zostera noltii* diminished rates of cannibalism (0.19 preys eaten per predator per day) when compared to low and medium densities of *Zostera* (0.38 preys eaten per predator per day). Our results suggest that *Carcinus maenas* daily mortality rates due to cannibalism seem to be independent from size up to instar 7 juveniles. In the case of Ria de Aveiro, the average daily mortality rates due to cannibalism (0.07) is approximately half of the mortality caused by pre-settlement processes (0.12).

Keywords: *Carcinus maenas*; cannibalism; *in situ* experiments; cages; prey size; predator size; refuge; *Zostera noltii*.

Introduction

Post-settlement events can greatly influence the survival and growth of newly settled individuals, causing significant differences between settlement and recruitment patterns (Ólafsson et al. 1994). The best documented cause of early mortality in post-settlement stages is, undoubtedly, predation, particularly in mobile species (Hunt and Scheibling 1997). Predation risk for young juveniles of many decapod species is dependent on the availability of shelter-providing habitats, such as seagrass and mussel beds, macroalgae, etc. (reviewed by Hunt and Scheibling 1997). In fact, structurally complex habitats strongly diminish predation risk, by providing refuges from predators (Heck Jr. et al. 2001, Moksnes et al. 2002) or by inhibiting predator search and capture of prey (Hovel and Lipcius 2002). Furthermore, in the case of vegetated habitats, the availability of food in these sites has been demonstrated to enhance growth and survival of juveniles (Perkins-Visser et al. 1996). Nevertheless, concentration of conspecific juveniles in a particularly nursery area can lead to intra- and intercohort competition for space and food (Iribarne et al. 1994) and in species which exhibit cannibalistic behaviour, early juveniles may be preyed upon by conspecifics of the same or older cohorts. In these circumstances cannibalism is thought to be an important factor affecting the size structure and abundance of successive settling cohorts, i.e., cannibalism acts as a density-dependent regulator of population size (Sainte-Marie and La France 2002). This is the case of several species of brachyuran crabs such as *Callinectes sapidus* (Hines and Ruiz 1995, Moksnes et al. 1997, Moksnes and Heck Jr. 2006), *Cancer magister* (Fernandez et al. 1993, Eggleston and Armstrong 1995), *Chasmagnathus granulata* and *Cyrtograpsus angulatus* (Luppi et al. 2001), *Chionecetes opilio* (Lovrich and Sainte-Marie 1997, Sainte-Marie and La France 2002), *Paralithodes camtschaticus* (Stevens and Swiney 2005) or *Carcinus maenas* (Moksnes et al. 1998, Moksnes 2004a).

The most obvious benefits of cannibalism are the acquirement of nutrients for maintaining metabolism during periods of low food availability of alternative food sources and the decrease of intraspecific competition for food (reviwed by

Luppi et al. 2001). Another potentially significant benefit of cannibalism is to enhance survival and reproduction prospects of related individuals by eliminating genetically unrelated individuals. In contrast, the most obvious costs of cannibalism are the risks associated with attacking a prey item that could retaliate back, the risk of transmission of host-specific diseases and parasites and the potential loss of inclusive fitness when a cannibal kills and consumes a genetically related individual (Schausberger 2003, Gallucci and Ólafsson 2007).

Among the factors that affect the intensity of cannibalism in decapod crustaceans are refuge availability, predator size and sex, predator assemblages, prey size, hunger of the predator, the availability of alternative food or the moult stage of the prey (reviewed by Luppi et al. 2001).

The shore crab *Carcinus maenas* (L.) is a dominant epibenthic predator who inhabits a variety of hard and soft intertidal and shallow habitats. It is native to Europe but has currently an extensive geographical distribution through artificial introduction to other continents (Grosholz & Ruiz 1995, Yamada et al. 2005). It holds a complex life history typical of marine invertebrates with a larval phase that includes 4 pelagical zoea stages, which develop on coastal waters, and a megalopal stage which reinvades of the estuaries or lagoonal nursery habitats. In the northwestern Portuguese coast, transport of *C. maenas* megalopae to the nearshore is accomplished by onshore advection following downwelling, southerly winds. Supply to the estuaries has a semilunar periodicity, during spring tides and occurs by selective tidal stream transport (more details in Queiroga et al. 2006). Settlement sites are usually intertidal vegetated habitats such as seagrass beds or other structured substrates (Moksnes 2002, Almeida and Queiroga 2003, Paula et al. 2006), which are actively selected by megalopae (Hedval et al. 1998, Moksnes and Wennhage 2001). Similarly to megalopae, young juveniles remain and inhabit those structured nursery areas, resulting in high densities of different size classes of recruits which can create mutual interferences that result in density-dependent growth, dispersal from refuge habitats (Moksnes 2002, Moksnes 2004b) and cannibalism (Moksnes 2004a). When juveniles attain sexual maturity they leave the intertidal area and

disperse to deeper habitats in a ontogenic habitat shift (Thiel and Dernedde 1994, Naylor and Kennedy 2003).

To out knowledge, estimates of in situ cannibalism rates have never been attempted in this species. This would be an important piece of information necessary to estimate mortality budgets and its allocation to the different phases in the species life cycle. In the present study, we conducted a field experiment with the purpose of examining cannibalism among juveniles of different size classes of the shore crab *Carcinus maenas*. We used cages deployed in the field, and, more specifically we analyzed (1) the size of the prey, (2) the size of the predator and (3) the presence of refuge (*Zostera noltii*) inside the cages on the survival of the preys.

Methods

Study system

The Ria de Aveiro estuary is located on the northwest coast of Portugal (40°38'N; 8°46W). It is a coastal shallow lagoon with a single narrow inlet of 400 m width that connects to the sea and it is classified as a bar-built estuary. Tides in the Ria de Aveiro are semi-diurnal, with a mean tidal range of 2.1 m at the inlet. This study was conducted in an intertidal mudflat of Canal de Mira (Fig. 5.1), one of the branches of the lagoon, at distance of 2.3 to 6.6 Km from the inlet. The experimental area where cages were deployed is characterized by sandy sediment with shells and it is located near one of the several seagrass *Zostera noltii* beds that can be found along the intertidal area of Canal de Mira. Ria de Aveiro harbours a commercially important population of the common shore crab *Carcinus maenas* (Gomes 1991).

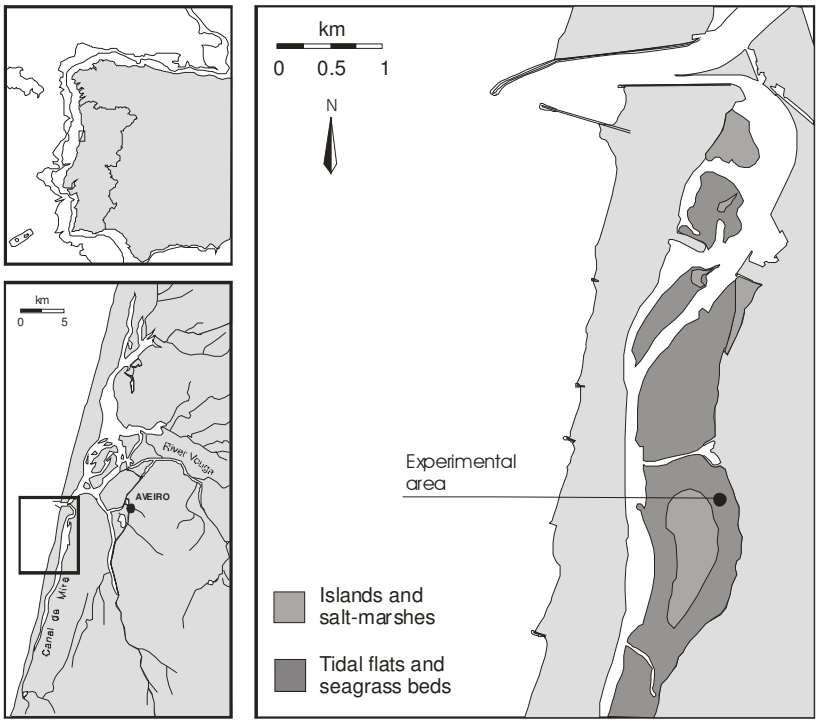

Figure 5.1. Location of the experimental area at Ria de Aveiro, where the cages were deployed.

Field experiments

The influence of cannibalism on the survival of *Carcinus maenas* juveniles was assessed by using cages that were deployed in the field. Cages were constructed with a wire frame, with the dimensions of 50 x 50 x 10 cm, supporting a plastic net with a mesh adequate to prevent juveniles to escape or intruders to enter. Care was taken to bury an extension of the lower rim of the plastic net into the sediment in order to avoid crabs or other mobile organisms to enter or escape the cage. Before setting up the cages, the sediment was carefully cleaned to ensure that there were no crabs buried inside. While cages were being prepared, shore crabs were hand captured in nursery areas located at the vicinity of the experimental area. These specimens were sorted according to pre-defined size classes and put in aerated buckets until the beginning of the experiments. We tested 2 sizes of prey juveniles: smaller preys, with ca. 5 mm carapace width (CW), corresponding to juvenile instars 3 and 4 and larger preys with 10 mm CW, corresponding to juvenile in instars at 5, 6 and 7 (Almeida et

al., 2008). Regarding the predators, 3 different sizes were tested: 15 mm, 20 mm and 25 mm CW. We also analyzed the absence of predator in cages in a control treatment.

The influence of refuge in cannibalism rate was tested by using patches of *Zostera noltii* that were transferred from a seagrass bed nearby the experimental site to the interior of the cages. We tested 3 densities of *Z. noltii*: low (covering ca. 5% of the cage area was covered), medium (about 20% of the cage was covered) and high (covering ca. 75% of the cage) densities. Regardless the density of *Zostera noltii* implanted inside the cage, at each cage 5 preys and 1 predator were tested. These numbers correspond to a density of 24 ind.m^{-2}, which are approximately two times the natural densities found in *Zostera noltii* seagrass at Ria de Aveiro (Almeida et al. *submitted*). When all the experimental conditions inside the cages were set up, the top of the cage was closed. Mortality was assessed after 72 h and each experimental condition was replicated 3 times.

Statistical analysis

Mortality was tested as the dependent variable in a 3-factor ANOVA where refuge (low, medium and high density of *Zostera*), prey size (5mm and 10 mm CW) and predator size (no predator, 15 mm, 20 mm and 25 mm CW) were the independent variables. Cochrane's tests performed before running the ANOVAs confirmed the assumption of homogeneity. *A posteriori* multiple comparison tests were carried out with the Student-Newman-Keuls (SNK) procedure.

Results

Our results show that, regardless of the cover of *Zostera*, smaller preys (ca. 5mm CW) were significantly more predated than larger preys (ca. 10 mm CW; SNK, $p < 0.05$; Table 5.1, Fig. 5.3). Moreover, predator size had a highly significant effect on mortality of juvenile preys (SNK, $p < 0.05$; Table 5.1). The

pos-hoc tests revealed that predators with 20 and 25 mm CW had a significant effect on mortality of smaller preys, at low and medium density of *Zostera* (SNK, $p < 0.05$; Table 5.1).

Mortality in the controls was always zero except in the case of small preys at high density of *Zostera*.

It is also interesting to notice that according to the ANOVA, the effect of refuge on the mortality of preys is nearly significant ($p = 0.0545$; Table 5.1). This circumstance is similar to the combination of the effects of refuge, prey and predator sizes ($p = 0.0614$; Table 5.1), indicating possible interactive effects between the main factors.

Table 5.1. *Carcinus maenas*. Results of the 3-way ANOVA testing the effect of Refuge (low, medium and high density of *Zostera*), prey size (5 mm and 10 mm CW) and predator size (control, 15 mm, 20 mm and 25 mm CW) on mortality of preys. *C*: Cochran's *C* statistic.

	dF	*ms*	*F*	*p*	
Refuge	2	1.85	3.1	0.0545	
Prey size	1	2.72	4.6	0.0379	*
Predator size	3	7.15	12.0	0.0000	***
Refuge x Prey size	2	0.26	0.4	0.6454	
Refuge x Predator size	6	0.66	1.1	0.3714	
Prey size x Predator size	3	0.76	1.3	0.2948	
Refuge x Prey size x Predator size	6	1.30	2.18	0.0614	
Residual	48	0.60			
			C=0.302; *ns*		

ns $p > 0.05$; * $p < 0.05$; *** $p < 0.001$

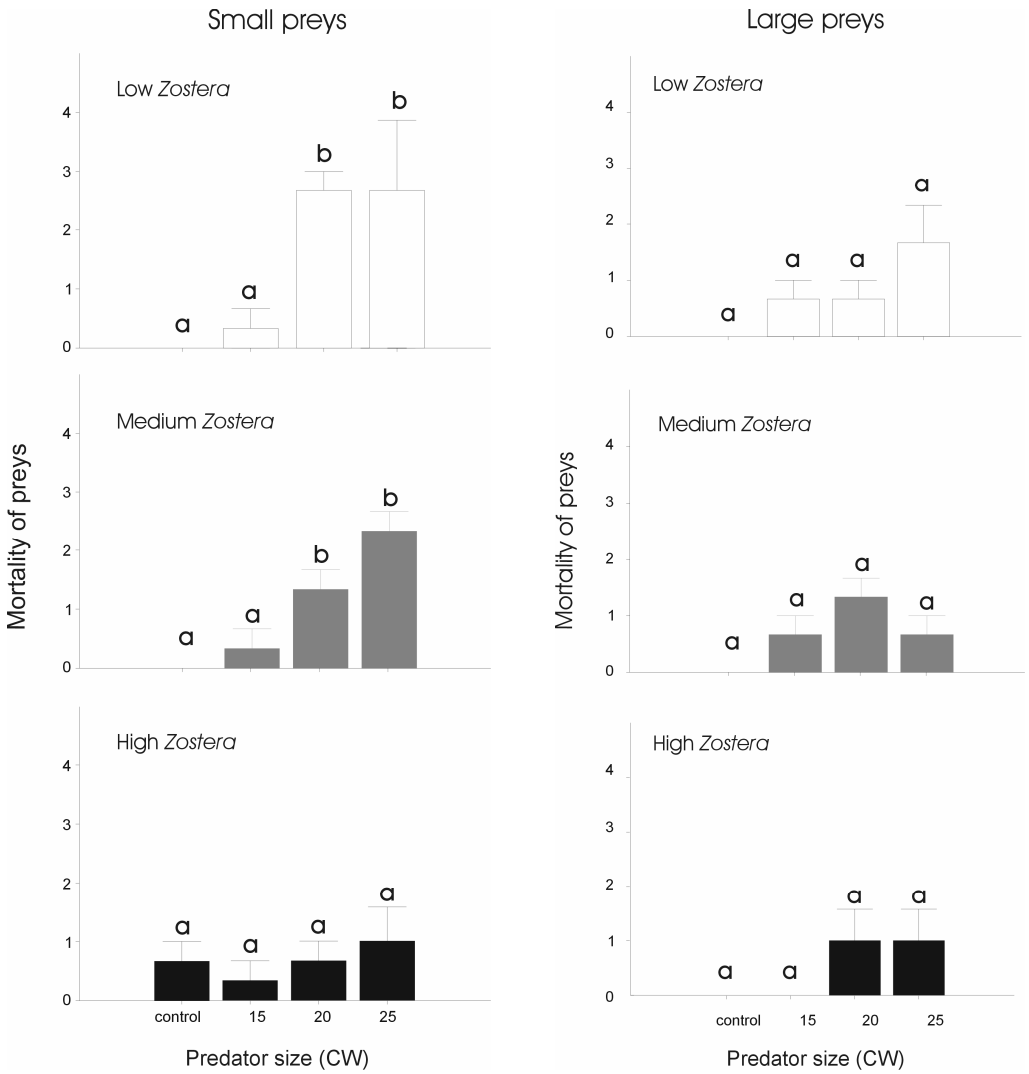

Figure 5.3. *Carcinus maenas*. Mortality on small (ca. 5 mm CW) and large (ca. 10mm CW) preys according to different predators (15, 20 and 25 mm CW), at different densities of *Zostera noltii*. Distinct letters above bars indicate statistically differences among groups (SNK; $p<0.05$).

Cannibalism rates ranged from 0 preys eaten per predator per day in the experimental conditions with large prey, small predator and high *Zostera* cover, to 0.89 preys eaten per predator per day in treatment with small prey, large or medium predators and low *Zostera* (Table 5.2).

Table 5.2. Mortality of preys, as number of preys eaten per predator per day, observed in the present study and in the experiments of Moksnes et al. (1997) and Moksnes (2004b). Densities of preys as prey per m^2. Zost: *Zostera*; meg: megalopae; Jx: instar x; CW: carapace width.

This experiment	*Carcinus maenas*				
Field				**Habitat**	
cage area: 0.25 m^2	**Preys**		Low	Medium	High
Predator: 15 mm CW	Density	instar	Zost	Zost	Zost
	20	J3-J4	0.11	0.33	0.11
	20	J5-J7	0.22	0.22	0.00
Field				**Habitat**	
cage area: 0.25 m^2	**Preys**		Low	Medium	High
Predator: 20 mm CW	Density	instar	Zost	Zost	Zost
	20	J3-J4	0.89	0.44	0.22
	20	J5-J7	0.22	0.44	0.33
Field				**Habitat**	
cage area: 0.25 m^2	**Preys**		Low	Medium	High
Predator: 25 mm CW	Density	instar	Zost	Zost	Zost
	20	J3-J4	0.89	0.78	0.33
	20	J5-J7	0.56	0.22	0.33

Moksnes et al. 1997	*Callinectes sapidus*			
Lab	**Preys**		**Habitat**	
tank area: 0.39 m^2	Density	instar	Sand	Grass
Predator: J9	102.0	meg	0.21	0.03
	23.0	J3	0.21	0.01
	7.7	J5	0.12	0.08

Moksnes 2004a	*Carcinus maenas*		
Lab	**Preys**		**Habitat**
tank area: 0.19 m^2	Density	instar	Mussel
Predator: J9	11	J1	0.00
	32	J1	0.30
	100	J1	2.00
	284	J1	3.90
	852	J1	7.90

Table 5.3. Post-settlement mortality daily rates due to cannibalism, observed in the present study. Zost: *Zostera*; Jx: instar x; CW: carapace width.

		Habitat		
Predator size	15 mm CW	**Low Zost**	**Medium Zost**	**High Zost**
Prey instar	J3-J4	0,02	0,06	0,02
	J5-J7	0,04	0,04	0,00

		Habitat		
Predator size	20 mm CW	**Low Zost**	**Medium Zost**	**High Zost**
Prey instar	J3-J4	0,18	0,08	0,04
	J5-J7	0,04	0,08	0,06

		Habitat		
Predator size	25 mm CW	**Low Zost**	**Medium Zost**	**High Zost**
Prey instar	J3-J4	0,21	0,15	0,06
	J5-J7	0,11	0,04	0,06

	Habitat		
	Low Zost	**Medium Zost**	**High Zost**
Average	0,10	0,08	0,04
Average all habitats	**0,07**		

Discussion

In this study we conducted a field experiment to assess mortality due to cannibalism in juvenile shore crabs at Ria de Aveiro. The overall results of our experiment showed that cannibalism rates in juvenile shore crabs increased with smaller preys, which were more predated than larger preys, with medium and large size juveniles being the most effective predators.

Studies addressing cannibalism among decapod juveniles typically focus on small preys as settlers (megalopae or glaucothoe) and first instar crab stage; e.g. *Callinectes sapidus* (Moksnes et al. 1997), *Carcinus maenas* (Moksnes 1998, 2004a), *Chasmagnathus granulata* and *Cyrtograpsus angulatus* (Luppi et al. 2001), *Chionoecetes opilio* (Sainte-Marie and Lafrance 2002) or *Paralithodes camtschaticus* (Stevens and Swimney 2005). All these studies reported very high

mortality among those instars. To our knowledge, studies reporting cannibalism on larger juveniles are not very common. Yet, it was possible to compare our observations with the ones obtained by Moksnes et al. (1997). In his experiments the author tested cannibalism upon juveniles of *Callinectes sapidus* at instar 3 (CW ranging from 4.3 to 5.8 mm) and 5 (CW ranging from 7.5 and 9.2 mm), by instar 9 predators (CW between 14.7 to 16.0 mm). Similarly to our results, smaller preys (megalopae and juvenile instar 3) were significantly more cannibalized than larger preys (juvenile stage 5). Although Moksnes experiments were conducted in the lab and tested a different portunid species, both prey and predator sizes are comparable to the sizes used in our experiment. We used data provided in Moksnes et al. (1997) to recalculate and standardize mortality rates in an attempt to compare results from the two studies (Table 5.2). It was interesting to notice that although concerning two different species, which inhabitate different nursery habitats and that were tested under different experimental conditions regarding cage size and overall density, mortality rates are similar. This can be seen by comparing Moksnes et al. (1997) experiment with juvenile instar 3 at a density of 23 individuals per m^2 with the experiments reported in the present paper for small predators with 15 mm CW.

Cannibalism upon megalopae and first instar juveniles of *Carcinus maenas* has also been studied by Moksnes (2004a) in laboratory experiments, using juveniles predators at instars 6 to 7 and densities similar to the ones found in the natural environment. This study concluded that cannibalism among juvenile shore crabs in nursery habitats was high and strongly density-dependent. Recalculated mortality rates obtained by Moksnes (2004a) are also given in Table 5.2. Comparing the second prey density tested by Moksnes (32 preys per m^2), which is analogous to the densities we tested in our trial (20 preys per m^2) we observe that mortality rates among these two conditions are similar.

The comparison of the present study to those of Moksnes et al. (1997) and Moksnes (2004a) allows two important generalizations. The first is that cannibalism rates among juveniles of portunid species inhabiting estuarine intertidal habitats are similar among different species at realistic prey and predator densities and shelter conditions, and when comparing preys and

predators of similar sizes. This indicates that similar size constraints related to food searching, agonistic behaviour and shelter likely apply to these species. The second is that, in the case of *Carcinus maenas*, daily mortality rates due to cannibalism seem to be largely independent from size up to instar 7 juveniles, or at least within the same order of magnitude.

Although not strictly significant at the 5% level, our results also suggest that, higher availability of refuge, i.e., higher densities of *Zostera noltii,* diminishes rates of cannibalism. Considering the trials conducted with higher refuge availability, the average estimate of mortality is 0.19 preys eaten per predator per day. At low densities of *Zostera* average mortality is 0.38 preys eaten per predator per day (Table 5.2). These results are consisting with literature, as the importance of shelter-providing habitats such as seagrass beds enhance survival in juvenile crabs, namely because cannibalism rates diminish significantly in such nursery habitats (Moksnes et al. 1997, Luppi et al. 2001, Stevens and Swimney 2005). Therefore, the variation in the distribution of seagrass habitats within Ria de Aveiro will have a direct effect on the recruitment.

Per capita average daily mortality rates (M) can be estimated from the geometric mean of survival rate using the following equation:

$$M = 1 - \left(\frac{N_t}{N_o} \right)^{\frac{1}{t}}$$

where t is time, N_0 is density at time 0, N_t is density at time t. From the abundance data provided by Queiroga et al. (1994) it is possible to estimate that abundance of megalopae inside the Ria de Aveiro is 100 times lower that the abundance of the newly hatched first zoea. Assuming a larval phase lasting in average 35 days (Nagaraj 1993) at the temperatures characteristic of late winter and spring in the Portuguese coast, the estimated daily larval mortality is 0.12. Considering the observations reported in the present study, in a 3 day experiments, it was possible to estimate mortality rates due to cannibalism (Table 5.3). The average daily mortality rates due to cannibalism generated was of 0.07, which is approximately half of the mortality caused by pre-settlement

processes. Considering that *Carcinus maenas* population at Ria de Aveiro is maintained by a low supply of larvae compared to similar species and ecosystems (reviewed by Queiroga et al. 2006), it is likely that larval supply may constitute a bottleneck on the regulation of this juvenile population, and that this is a population pre-settlement regulated. This result contrasts with the conclusion of Moksnes (1999) that established the juvenile population of shore crab at Sweden as population regulated mainly by post-settlement processes, where density-dependent inter-cohort cannibalism and interference competition were considered the mechanism creating a predation mediated shelter-bottleneck at settlement.

Finally, it is important to stress the importance of conducting experiments under natural field conditions on early life stages of marine benthic species. Their use improves our understanding on the influence of not only cannibalism, but also competition, predation, movements and other demographic processes, which are needed to understand population dynamics of these species.

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Chapter 6

Final remarks

Final remarks

The main objective of this thesis was to understand the relative importance of pre- and post-settlement processes in the control of the population density of the crab *Carcinus maenas* in two Portuguese estuaries and contribute to the improvement of conceptual and predictive models for recruitment and population regulation in this species. Temporal patterns of megalopae settlement, juvenile distribution as a function of habitat, juvenile growth rates and cannibalism among different juvenile size classes were assessed using a combination of methodologies that were used in various field experiments, namely plankton nets (Chapter 2), artificial settlement substrates (Chapters 2 and 3), benthic suction sampling and pitfall traps (Chapter 4) and controlled caging experiments (Chapter 5).

Abundances of *Carcinus maenas* megalopae in Ria de Aveiro were low, not only compared to megalopal abundances at Rio Mira estuary (ca. 18 times lower) but also in relation to abundances of the same and other portunid species in other systems (ca. 1 to 3 orders of magnitude; Olmi 1994, van Montfrans et al. 1995, Moksnes and Wennhage 2001). These observations suggest a low level of larval availability and supply in the northern Portuguese coast. Transport of *C. maenas* megalopae to the nearshore followed a fortnightly cycle with maximum larval supply at the time of maximum tidal amplitude. This transport was enhanced by southerly downwelling winds. The immediate response of supply of *Carcinus maenas* megalopae to the amplitude of the tide is consistent with the immediate responses of megalopae to hydrostatic pressure, salinity and turbulence during selective tidal stream transport. Shore crab maximum settlement in Ria de Aveiro occurred around neap tides, indicating that settlement was clearly decoupled from supply.

Densities of *Carcinus maenas* juveniles in both estuaries followed the pattern of megalopal abundances: at Ria de Aveiro juvenile abundances were 5 to 15 times lower than at the Rio Mira estuary. In relation to other systems, namely at Sweden, densities are even lower (Moksnes 2002). Our measurements at Ria de Aveiro estimated an average duration of juvenile instar of about 1 wk

and a 41 d total time to reach instar 6. Growth rates were variable according to crab stage, ranging from 0.09 (instar 2) to 0.28 mm per day (instar 6).

We observed an obvious temporal separation of locomotory activity between smaller and larger juveniles: while small juveniles were more active during dawn and dusk, large juveniles showed higher locomotion during the night and day. Moreover, movement of large juveniles was shown to be very tenuous in dense *Zostera* patches, but very high in sparsely vegetated areas; therefore, small juveniles are relatively protected under dense vegetation cover due to lower mobility of larger crabs. This temporal segregation of activity windows between juvenile crabs of different size, may act as a key mechanism to reduce cannibalism and subsequently increase carrying capacity of nursery habitats.

Finally, we observed high daily mortality rates due to cannibalism in experiments conducted *in situ* and at natural densities. Smaller preys were more predated than larger preys, but our results have shown that mortality rates due to cannibalism among *Carcinus maenas* juveniles seemed to be largely independent from size up to instar 7. In conditions of high availability of refuge, cannibalism rates were lower than at low and medium densities of *Zostera* (0.05 preys eaten per predator per day in opposition to 0.09 preys eaten per predator). Our studies estimated an average daily mortality rates due to cannibalism of about half of the average daily mortality caused by pre-settlement processes (0,07 and 0,12, respectively). Given the low larval supply of shore crab larvae into Ria de Aveiro, it appears that this is a pre-settlement regulated population.

Although seagrass beds of *Zostera noltii* were not specifically studied in our experiments, the work conducted during this thesis have highlighted their importance as nursery habitats, both at Ria de Aveiro and at the Rio Mira estuary. This habitat has shown to be of great importance in the survival and recruitment of the *Carcinus maenas* juvenile population, namely as a preferential megalopal settlement habitat and in diminishing daily juvenile cannibalism rates.

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